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# CHARACTERISTICS OF FOREST SOILS OF THE NORTHWESTERN UNITED STATES

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A large part of the farm land in the northern humid, temperate climates was developed under forest cover. This is true of much of the land now under cultivation in the Northwest. A large part of the Northwestern States is most suitable for forest production permanently. Approximately one-third of Oregon is commercial forest and in the Northwest there occur some of the finest forest soils in the world.

A recent investigation by the author, of organic soil colloids separated from peaty soil profile layers, indicates unusually high base exchange capacity in mixtures of organic and inorganic soil colloid. In forest soils the organic litter, fermenting layer, humified layer, and soil humus layer of different forest types should afford favorable material for studying the trend of humification, reaction, base capacity, and related characteristics. Here every degree of decomposition and also a range of natural mixtures of organic and inorganic constituents can be obtained. The cycle of transformations of organic constituents is now recognized as an outstanding factor in forest soil fertility.

Very little exact data are available on forest soils, particularly of the Northwest, or the effects of forest organic matter on soil fertility, yet foresters recognize the fundamental rôle of the organic matter in forest soil fertility. Information is also needed as to the best methods of improvement of newly cleared logged-off land where location, soil, and topographic conditions are attractive for crop production. An investigation has therefore been undertaken to study the significance of base exchange and other characteristics of typical Northwestern soil profile layers, and results thus far obtained are reported herein.

It is interesting to note that Gedroiz in 1922 recognized the fact that humic bases as well as zeolitic bases are concerned with base absorption. Since he was not able to separate these he assumed that the humic portion behaves similarly to the zeolitic. Recently Albin (1), McGeorge (5), and the writer (9) have shown that peat soils possess high base exchange capacity; in the case of organic soil colloids this has been found to be especially great and appears to reside in the lignin fraction (9). Decomposition results in a higher concentration of lignin and a consequent increase in base exchange capacity.

<sup>1</sup> Published as technical paper No. 176, with approval of director, Oregon Agricultural Experiment Station. Contribution from department of soils.

## EXPERIMENTAL

The plan followed in this study has been to collect profile layer samples from typical forested areas for use in laboratory determinations. Nine soil profiles were collected, including three from the coast region, three from the Willamette water shed, and three from eastern Oregon, representing redwood (*Sequoia sempervirens* [Lamb] Anal.), yellow pine (*Pinus ponderosa*, Douglas), Douglas fir (*Pseudotsuga taxifolia* [Lamb] Britt), and oak (*Quercus Carryana*, Douglas) land. Samples were taken of the litter, fermenting layer, fermented layer, soil-humus layer, the leached eluvial inorganic soil layer, and the illuvial or B horizon, which is the zone of accumulation. In some cases a sample of underlying soil material was obtained. Podzolic, lateritic, and brownerth soil types are included.

Determinations made and methods used thus far include (a) colorimetric determination of reaction value, or pH; (b) adsorption of moisture vapor over 3.3 per cent sulfuric acid; (c), total nitrogen by the Kjeldahl method;<sup>2</sup> organic matter, according to Rather (7); and base exchange capacity using barium chloride with some comparisons of the use of ammonium chloride.

For base exchange determinations 1-gm. or 2-gm. samples were placed in tall 250-ml. pyrex beakers provided with short Pasteur-Chamberlain filter candles and connected to suction flasks. Treatments were repeatedly made with 2 per cent hydrochloric acid, then with neutral half-normal barium chloride. After the samples were washed with warm distilled water until free of chlorides, the barium was displaced with the half-normal neutral ammonium chloride, precipitated and weighed as barium sulfate. As a check, one dozen samples were leached free of chlorides after displacement with the ammonium chloride, and the ammonia was recovered by adding magnesium oxide and distilling at low heat into a saturated solution of boric acid for titration in which brom-phenol blue was used as indicator.

*Comparison of methods*

A comparison of determinations of base exchange capacity by these two methods is shown in figure 1. Even with careful heating there may be a tendency for nitrogen compounds to decompose during distillation, so that the organic layers of the forest soil profile tend to give slightly higher values with the ammonium method. However, results are in general agreement. The barium chloride treatment is convenient for making a large number of determinations and only a slight tendency toward decomposition was found where successive determinations on the same sample were made.

*Physical and microörganic characteristics of forest soil profiles*

The forest soil profile samples are briefly described in tables 1 and 2, in which the leading types of vegetation, character of litter, and thickness of layers or

<sup>2</sup> Organic matter determinations were made by Dr. R. E. Stephenson and total nitrogen determinations were made in duplicate by Professor E. F. Torgerson, of these laboratories.

horizons are indicated. These forest soils may be characterized as being generally of a friable, mellow structure, and usually having good drainage. Although the type of vegetation materially affects the soil development, the influence of climate has appeared to have a more profound effect under the conditions studied.

After notes had been taken on visible physical characteristics, the soils were air-dried, ground to pass a screen with 2-mm. perforations, and were then stored in fruit jars.

Absorptiveness for moisture vapor was determined with 1-gm. samples over 3.3 per cent sulfuric acid under standard conditions described by Robinson (8). Absorptiveness usually reaches the maximum in the fermenting layer. A secondary maximum of lesser magnitude occurs in the B horizon or zone of accumulation of the subsoil. Absorptiveness indicates colloidity. Pure inorganic soil colloid is known to absorb approximately 30 per cent moisture;

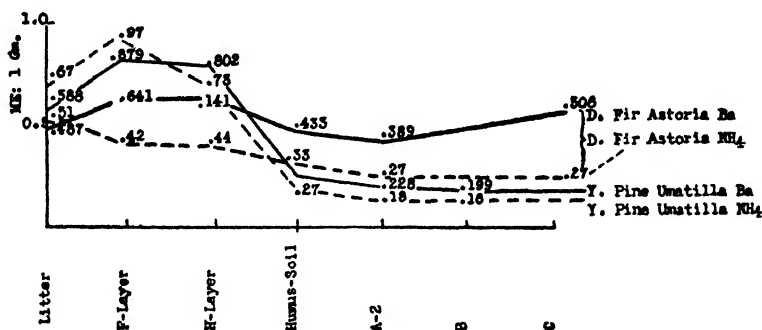


FIG. 1. BASE EXCHANGE CAPACITY OF TWO FOREST SOIL PROFILES  
Ba vs.  $\text{NH}_4$  method

it has been recently shown (9) that organic soil colloid of low ash content absorbs nearly 45 per cent. The Meyer clay colloid absorbed 27.26 per cent and the peat colloid 40.01 per cent moisture. Perhaps other volatile matter was lost from litter upon drying at  $100^\circ\text{C}$ .

Fresh moist samples from profile 3 taken near Astoria were examined and counts made.<sup>3</sup> Results indicate that the maximum number of microorganisms occurs in the fermenting layer. More bacteria and fewer molds were found in the litter.

#### *Chemical characteristics of forest soil profiles*

*Soil reaction.* Colorimetric determinations of reaction value (or pH) of samples from nine forest soil profile layers for which results are given (table 2), show high acidity in the organic layers under conifer forests and conditions of heavy precipitation. The organic acids from conifer litter have caused

<sup>3</sup> By Dr. W. B. Bollen, soil bacteriologist, Oregon Agricultural Experiment Station.

TABLE 1  
Absorption of moisture by forest soil profile samples  
(Over 3.3 per cent sulfuric acid)

Locality.....	Smith Riv- er, Calif.	Bandon, 4- mile Cr.	Astoria Exp. Sta.	Gresham, 2 mi., S. C.	McKenzie Bridge, 3 mi. E.	Corvallis, 1 mi., S. W.	Sisters, 4 mi. W.	Wanoga Butte	Pioneer Camp, Blue Mts.
Cover.....	Redwoods	Pine and Fir	Douglas Fir	Douglas Fir	Douglas Fir	Oak	Yellow Pine	Yellow Pine	Pine and Fir
Diameter feet.....	3-6	1	1-2	1-2½	1-2½	1-2	1-2	1	1-3
Soil Type.....	Gray- Brown Fine Sandy Loam	Podzolic Very Fine Sandy Loam	Lateritic Silt Loam	Powell Silt Loam	Gravelly Silt Loam	Carlton Silty Clay Loam	Light Me- dium Silty Loam	Light Me- dium Loamy Silt	Brown Silty Loam
Absorption—moisture (per cent) in:									
Litter.....	20.10	19.70	33.31	19.66	25.50	24.20	23.19	34.30	21.09
F-layer.....	25.88	23.80	13.10	21.11	21.00	31.50	21.45	23.30	24.77
H-layer.....	23.60	12.80	18.00	12.51	18.50	15.50	10.03	8.70	5.06
H-soil.....	14.80	7.69	12.00	6.27	11.50	7.70	4.41	4.20	8.29
A <sub>1</sub> horizon.....	6.70	6.30	8.40	6.12	10.30	7.79	9.01	21.30	10.01
B horizon.....	4.60	2.50	.....	4.11	10.30	.....	.....	3.63	5.71
C horizon.....	.....	.....	14.5	.....	.....	.....	.....	.....	.....

TABLE 2  
*Characteristics of Northwestern forest soil profiles*

SOIL PROFILE LAYERS—DEPTH	pH	BASAL EXCHANGE CAPACITY ME:1 GM.	ORGANIC MATTER* <i>per cent</i>	TOTAL† NITROGEN
<i>Cost section</i>				
1. Redwoods—Smith River, California, 1 mile below steel bridge				
Decayed redwood log.....	5.8	0.530	95.58	0.201
0-2 inches litter, needles, etc.....	6.3	0.411	88.96	0.854
2-5 inches F layer.....	6.7	0.372	58.26	0.623
5-6 inches H layer.....	6.8	0.156	32.84	0.483
6-12 inches (humus) and soil, fine sandy loam.....	6.9	0.206	7.76	0.224
12-24 inches gravelly silt loam.....	6.9	0.094	2.81	0.094
2. Blacklock—Podzolic soil 4 miles South of Bandon, Oregon. Pine and fir				
0-2 inches litter, needles, moss, etc.....	6.3	0.334	73.10	1.325
2-2½ inches F layer.....	6.1	0.336	33.60	0.946
2½-3 inches H layer.....	6.1	0.271	25.10	0.669
4-12 inches bleached, very fine sandy loam.....	6.1	0.106	2.74	1.032
16-20 inches iron pan.....	6.0	0.060	1.09	1.252
20-30 inches raw soil material.....	6.3	0.034	0.18	0.037
3. Douglas fir—Lateritic soil, Astoria Experiment Station Grove				
0-½ inches litter.....	4.4	0.487	88.10	0.989
½-1 inches F layer.....	4.8	0.641	80.50	1.119
1-1½ inches H layer.....	5.0	0.641	31.80	0.655
1½-2½ inches humus soil, silt loam ..	5.1	0.433	7.08	0.293
2½-14 inches Soil ..	5.0	0.389	3.13	0.155
60-66 inches C layer.....	5.0	0.506	1.30	0.057
<i>Willamette section</i>				
4. Douglas fir—Powell silt loam 2 miles South East of Gresham, Oregon				
0-½ inches fir needles, twigs, fern, etc....	5.4	0.175	90.37	1.006
½-1 inches F layer.....	6.5	0.166	75.62	1.059
1-2 inches H layer.....	6.7	0.048	40.86	0.532
2-4 inches humus soil, silt loam.....	6.8	0.041	14.52	0.136
4-12 inches silt loam.....	6.9	0.024	2.07	0.099
24-36 inches B layer, silt loam.....	7.0	.....	0.72	0.038
5. Douglas fir—3 miles above McKenzie Bridge, Oregon. Fir up to 30 inches				
0-½ inches litter, needles, twigs, moss....	5.4	0.535	67.80	0.894
½-1½ inches F layer.....	6.5	0.715	37.20	0.938
1½-3 inches H layer.....	6.6	0.591	38.20	0.728
3-4 inches humus-soil—gravelly silt loam	6.7	0.101	3.23	0.186
4-18 inches A 2.....	6.8	0.031	1.53	0.099
30-36 inches B.....	6.9	0.038	0.81	0.060

TABLE 2—*Concluded*

SOIL PROFILE LAYERS—DEPTH	pH	BASAL EXCHANGE CAPACITY ME:1 GM.	ORGANIC MATTER*  per cent	TOTAL† NITROGEN
Willamette section—Concluded				
6. Oak—up to 2 feet, on brownerth near Corvallis, Oregon				
0- ½ inches litter. . . . .	6.3	0.300	88.50	1.212
½- ¾ inches F layer. . . . .	6.6	0.449	83.85	1.373
¾-1½ inches H layer, mouldy. . . . .	6.6	0.446	58.88	1.117
2-4 inches humus soil, silty clay loam ..	6.8	0.254	11.56	0.374
4-12 inches silt loam . . . . .	6.7	0.144	1.75	0.127
Eastern Oregon				
7. Yellow pine—3 miles west of Sisters, Oregon				
Yellow pine up to 22 inches				
0- ½ inches pine needles, chemisa, and grass . . . . .	4.7	0.380	93.90	0.543
½-1 inches F layer . . . . .	5.2	0.447	26.70	0.735
1-1½ inches H layer . . . . .	6.3	0.240	24.20	0.428
1½-4 inches humus soil, medium sandy loam . . . . .	6.9	0.115	4.37	0.356
12-30 inches B (8 per cent gravel) . . . . .	6.7	0.062	1.59	0.384
8. Yellow pine—Wanoga Butte, Oregon, 20 miles South West Bend, Oregon				
0- ½ inches needles, etc. . . . .	4.5	0.533	97.00	0.876
½-3 inches F layer . . . . .	5.8	0.973	92.78	1.075
3-3½ inches H layer . . . . .	6.8	0.655	41.40	0.434
4-6 inches soil and humus, medium loamy silt. . . . .	6.9	0.355	3.88	0.067
6-18 inches pumice soil and roots. . . . .	6.9	0.248	2.95	0.044
24-36 inches raw pumice . . . . .	7.0	0.283	1.60	0.030
9. Yellow pine and fir—½ mile South West of Pioneer Camp, Oregon, Trail Hiway, Umatilla County, Trees 1-3 feet in diameter				
0-1 inches needles and twigs, etc. . . . .	6.8	0.588	77.20	1.060
1-2 inches F layer . . . . .	6.8	0.879	69.10	1.177
2-2½ inches H layer . . . . .	6.8	0.802	43.70	0.861
2½-10 inches humus—soil brown silty loam . . . . .	6.9	0.313	3.72	0.179
10-24 inches B 1. . . . .	6.9	0.228	1.37	0.092
24-40 inches B 2, form on rock at 40 inches. . . . .	7.0	0.199	0.78	0.059

\* Determined by R. E. Stephenson.

† Determined by F. F. Torgerson.

removal of bases from the upper layers of the conifer forests to a greater extent than under deciduous forest cover, which gives less acid litter, having a higher ash content. The more nearly neutral reaction is found in the B layer or zone

of accumulation and the deep subsoil samples are nearly neutral in reaction after the slightly modified soil material of the C horizon is reached.

*Organic matter content.* The organic matter content of profiles studied decreases with depth and even the litter shows evidence of contamination with inorganic material. Yellow pine needles in the litter of profile 8 show only 3 per cent inorganic matter by the methods used. The Douglas fir litter of profile 5 was nearly one-third inorganic matter. A sample of the deep subsoil or C horizon of profile 3 from near Astoria, taken at a depth of over 5 feet, was below the visible influence of roots and contained 1.3 per cent organic matter, whereas the raw soil material from below the iron pan in the podzol profile, No. 2, contained but 0.18 per cent.

*Total nitrogen.* Determinations of total nitrogen show the maximum nitrogen content to occur usually in the fermenting layer. It appears that the nitrates formed in the early stages of decomposition are assimilated by and temporarily stored in the bodies of the microorganisms. Thus in this F layer moulds seemed to be more or less prevalent and were especially abundant in the oak soil profile, No. 6. The indicated nitrogen-carbon ratio is rather wide, especially in the case of conifer forest soils. This is related to the more inert nature of the conifer litter which is unfavorably low in nitrogen and earthy bases for prompt decomposition.

*Base exchange capacity.* Base exchange capacity is reported in millequivalents per gram sample. It is interesting to note that the nearly fresh litter of various forest types manifests base exchange capacity. The maximum base capacity is usually found in the fermenting layer or where there is a mixture of about three-fourths humified organic matter with one-fourth inorganic material. The value is only slightly lower in the humified layer as a rule. Forest profile samples from the semi-arid sections of eastern Oregon have been less leached and show higher base capacities in their surface layers than do those from the humid coastal region where leaching and some degeneration have occurred.

#### *Base exchange capacity of artificial mixtures*

To study further the effects of mixtures on base exchange capacity, organic soil colloids isolated from neutral peat soil of low ash content were mixed with colloids derived from Aiken silty clay loam. These materials were mixed in proportions varying by 20 per cent intervals, with resulting base capacities as presented in figure 2. With this mixture the maximum base exchange capacity was realized with 80 per cent peat and 20 per cent clay.

In a second experiment, using the same peat colloid in mixtures with colloid from Meyer clay adobe from near Medford, a maximum base capacity was obtained with 60 per cent peat and 40 per cent clay colloid. The Meyer clay colloid had a reaction value of pH 8, or was slightly alkaline, and had a much higher base exchange capacity when used alone than did the acid Aiken colloid (pH 6.2). There is a striking similarity between the base exchange capacity



of natural mixtures such as occur in sedimentary peat layers or in the humified layers of forest soil profiles and that of these artificial mixtures, as will be noted by comparing figures 1 and 2.

The author recently studied the effect of mixing peat colloid with clean quartz sand, using 0.1 per cent, 0.5 per cent, and 1 per cent proportions of peat colloid. The base exchange capacity per gram of colloid increased where it was more thinly spread over the sand grains. The experiment was repeated using lignin isolated from fiber flax with similar results. These experiments (9) indicate that the base exchange activity may increase in the case of lignin down to one oriented molecular layer. Great base exchange capacity has also been found in connection with these studies of organic colloid derived from decomposing sweet clover straw in the presence of lime and mixed with inorganic soil colloid.

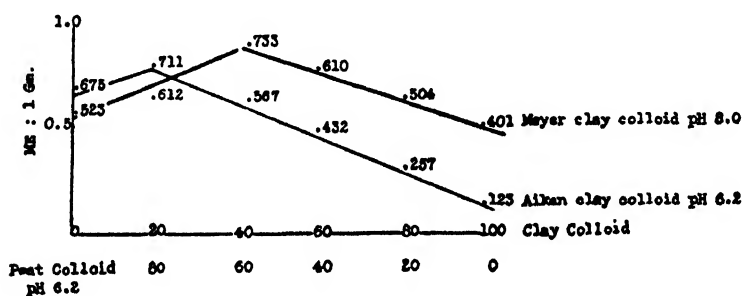


FIG. 2. BASE EXCHANGE CAPACITY OF PEAT AND CLAY COLLOID MIXTURES

#### DISCUSSION

The evidence herein presented is to the effect that a mixture of organic soil colloid and clay colloid has a very high base adsorbing capacity. This correlates with the high base exchange capacity of sedimentary peat layers and humifying layers of forest litter and brings out the fundamental rôle of organic soil colloids in soil conservation. The activity of earthworms in neutral brown earths or in humified layers of deciduous forest soils aids in the formation of an intricate mixture of organic and inorganic colloidal matter. The nitrogen is concentrated in the organic colloid fraction. Lunt (4) has recently reported finding that approximately 40 to 45 per cent of the exchange calcium in the whole forest soil profile was in the humic portion and that as much as 61 per cent of the total calcium in the humified layer was in exchange form. In other words, as the lignin concentration increases, the exchange portion of the calcium increases.

It appears to be easier to build up and keep up base exchange capacity of a forest soil that is well supplied with calcium, contains some deciduous growths (11), and is reasonably free from leaching. This helps explain the occurrence of black earth in northern semi-arid continental prairies. The presence of

lime facilitates building up of organic matter in arid soils when brought under irrigation and rotated with legumes, as recently noted (10). Decaying organic matter seems to increase nutrient supplying power of soil as to phosphate (6), iron (2), and potassium (3), as well as other bases and also nitrate.

The forest litter helps maintain tilth and conserve moisture with no cultivation. Acid conifer litter is resinous, low in nitrogen, and also slow to decay. It yields an acid extract which leaches out the bases, giving rise to the bleached gray, podzolic layer. These bases are removed partly in colloidal solution and carried down to a zone of more neutral reaction where precipitation occurs. The humus is believed to form a protecting cover to colloidal iron and hold it in colloidal solution during removal to the lower layer where it precipitates and helps form the hardpan. The analyses show some accumulation of organic matter in the coffee-brown pan formation of the Blacklock soil.

Logged off land in the humid Northwest is often acid and in need of lime, which will precipitate or render toxic substances unharmed. Decaying manure reinforced with superphosphate may also be expected to favor a reasonable rate of decomposition and increased productiveness.

Results obtained should be of value in economy of soil organic matter and conservation of base exchange capacity or protection against soil degeneration. The high nutrient supplying power of the humified layers of forest soils is further supported by the mass of feeding roots observed therein. The character and amount of forest litter and the trend of humification of this material under the climatic conditions obtaining have a profound effect upon soil development and continued soil productiveness.

#### SUMMARY

The great forest soils of the Pacific Northwest have been little studied. Their organic layers yield materials at different stages of decomposition and with different proportions of mineral earth intermixed. Studies made with nine forest soil profile layers usually show maximum absorptiveness for moisture vapor or colloidalness in the fermenting layers. Here the maximum numbers of microorganisms were also found. The maximum base exchange capacity was generally found in nearly neutral fermenting or largely humified organic layers containing approximately 75 per cent organic matter. A similar type of adsorption curve was obtained whether barium or ammonium ion was the saturating agent. Artificial mixtures of organic and inorganic soil colloids varied by 20 per cent interval, show a corresponding base adsorption curve. That surface concentration may help account for this was recently suggested in connection with similar results obtained with natural colloids separated from sedimentary peat layers (9). Marked activity of earthworms in neutral or calcareous soil abundantly supplied with decaying ligneous organic matter favors formation of this desirable intimate mixture of organic and inorganic soil colloids. Feeding roots are massed in the forest soil just below this organic

layer. These results should be of value in composting, in the use of litter or green manure, as well as in conservation of base exchange capacity of soil against degeneration.

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# THE DISTRIBUTION OF POTASSIUM IN DECIDUOUS ORCHARD SOILS IN CALIFORNIA

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The scorching of foliage of prune (*Prunus domestica* var. Agen) trees in parts of the Sacramento Valley seems to be associated to some extent with the potassium content of the soil. It seemed desirable to study the potassium relationships as they exist in various depths in areas where scorching does not appear and to compare these with soils in which prune trees show this trouble.

Analyses of soils in 21 prune orchards<sup>2</sup> in various sections of the state have, therefore, been made in the pomology laboratory at the University Farm, Davis. The approximate locations of these orchards and the representative soil types are listed in table 1. The soils are all of alluvial origin and exhibit no marked changes in texture in the top 4 feet.

It soon became evident from the analyses of these soils that the water-soluble and replaceable potassium varied with the depth of sampling. A definite reduction in these two potassium fractions with increased depth of sampling has been found in practically all the soils studied to date. The data listed in table 2 are typical of the changes in the water-soluble potassium. The reduction is most marked in the soils having the higher amounts. Although a great variation exists in the water-soluble potassium in the surface foot of the various orchards, the range of concentrations is narrowed with increased depth.

A study of table 3 reveals that the reduction in replaceable potassium in the deeper soil is marked, but not quite so rapid as that reported for the water-soluble form. Soils from the Los Molinos 3 and 4 and Vina 1 orchards seem to maintain their high concentrations of replaceable potassium in the third and fourth feet to a greater degree than one would be led to expect from the water-soluble fraction of these soils. The relationship between water-soluble and replaceable potassium does not seem to be one of simple hydrolysis, as suggested by Magistad (4). Samples identical in their replaceable potassium content but taken at varying depths exhibit varying contents of water-soluble potassium. Generally the surface samples show proportionally a greater water-soluble fraction than do the deeper samples.

<sup>1</sup> I am indebted to Mr. C. J. Hansen and Mr. E. A. Wilkins for their assistance with the chemical analyses.

<sup>2</sup> Subsequent data have been obtained from 10 additional orchards and are in accord with the results presented here.

TABLE 1  
*The prune orchards in which soil samples were studied*

ORCHARD	COUNTY	OWNER	SOIL TYPE
1. Los Molinos 1.....	Tehama	Lindauer	Sacramento fine sandy loam
2. Los Molinos 2.....	Tehama	Mundorff	Sacramento silty clay loam
3. Los Molinos 3.....	Tehama	Smith	Sacramento silt loam
4. Los Molinos 4.....	Tehama	Barton	Sacramento silty clay loam
5. Vina 1.....	Tehama	Woolsey	Vina fine sandy loam
6. Richfield 1.....	Tehama	Aasen	Elder silt loam
7. Chico 1.....	Butte	Canfield	Farwell fine sandy loam
8. Chico 2.....	Butte	McVay	Farwell loam
9. Chico 3.....	Butte	Peters	Farwell loam
10. Chico 4.....	Butte	Sypher	Vina fine sandy loam
11. Colusa 1.....	Colusa	Wolfin	Sacramento silty clay loam
12. Colusa 2.....	Colusa	Taft	Sacramento silty clay loam
13. Grimes 1.....	Colusa	Faxon	Sacramento fine sandy loam
14. Davis.....	Yolo	Univ. Farm	Yolo loam
15. Gilroy 1.....	Santa Clara	Barbari	Yolo silt loam
16. Paradise Valley 1.....	Santa Clara	Ward	Vina gravelly loam
17. Morgan Hill 1.....	Santa Clara	Hansen	Yolo silt loam
18. Saratoga 1.....	Santa Clara	Anderson	Yolo gravelly loam
19. Cupertino 1.....	Santa Clara	Wiesendanger	Yolo clay loam
20. Berryessa 1.....	Santa Clara	Moody	Yolo gravelly clay loam
21. Saratoga 2.....	Santa Clara	Payne	Yolo loam

TABLE 2  
*Water-soluble (1:1) potassium content of dry soil in relation to the depth of sampling*

ORCHARD	WATER-SOLUBLE POTASSIUM			
	First foot	Second foot	Third foot	Fourth foot
	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
1. Los Molinos 1.....	2 6	1 6	1 4	1 4
2. Los Molinos 2.....	5 6	2 8	2 2	.. .
3. Los Molinos 3.....	12 0	6 3	4 6	3 7
4. Los Molinos 4.....	10 4	8 8	8 3	9 0
5. Vina 1.....	8 0	10 4	8 2	.. .
6. Richfield 1.....	10 0	6 2	3 5	1 5
7. Chico 1.....	1 2	0 4	0 4	0 2
8. Chico 2.....	2 1	0 7	0 9	0 8
9. Chico 3.....	6 7	2 1	1 2	0 8
10. Chico 4.....	16 3	10 8	5 8	1 8
11. Colusa 1.....	4 3	2 3	2 3	1 5
12. Colusa 2.....	1 9	0 9	1 7	1 0
13. Grimes 1.....	49 5	49 8	40 6	14 3
14. Davis.....	5 3	2 2	1 2	1 2
15. Gilroy 1.....	13 7	5 2	1 7	1 2
16. Paradise Valley 1.....	5 3	3 0	2 3	1 9
17. Morgan Hill 1.....	7 4	1 6	0 9	0 8
18. Saratoga 1.....	9 7	5 7	2 7	2 0
19. Cupertino 1.....	14 2	9 0	2 8	1 1
20. Berryessa 1.....	7 2	3 3	1 5	1 7
21. Saratoga 2.....	6 6	2 0	1 0	1 2

Determinations of "active" potassium in these soils using 0.2 *N* nitric acid as described by Fraps (1) give results not far different from those obtained by replacement with the ammonium ion. A comparison of a few values in table 4 led to the conclusion that the replaceable and active fractions differed very slightly and that a continued study by this method would only be a duplication of the ammonium acetate extraction.

It was thought that such concentration gradients as were found in the water-soluble and replaceable potassium contents might be proportionally reflected in the analyses for total potassium. Fusion analyses of representative soils

TABLE 3  
*Replaceable potassium content of dry soil in relation to depth of sampling*

ORCHARD	REPLACEABLE POTASSIUM			
	First foot	Second foot	Third foot	Fourth foot
	<i>p p m</i>	<i>p.p.m.</i>	<i>p p m.</i>	<i>p.p.m.</i>
1. Los Molinos 1.....	170	140	140	130
2. Los Molinos 2.....	135	88	51	..
3. Los Molinos 3.....	650	405	342	350
4. Los Molinos 4.....	661	574	491	426
5. Vina 1.....	437	356	349	334
6. Richfield 1.....	173	120	105	90
7. Chico 1.....	110	86	84	52
8. Chico 2.....	134	84	74	49
9. Chico 3.....	434	244	128	97
10. Chico 4.....	985	950	497	183
11. Colusa 1.....	293	138	118	100
12. Colusa 2.....	141	102	103	116
13. Grimes 1.....	1,240	1,180	1,027	489
14. Davis.....	325	180	153	124
15. Gilroy 1.....	461	270	143	119
16. Paradise Valley 1.....	341	174	175	143
17. Morgan Hill 1.....	383	221	168	142
18. Saratoga 1.....	355	171	150	140
19. Cupertino 1.....	625	466	259	140
20. Berryessa 1.....	268	135	116	126
21. Saratoga 2.....	284	144	108	92

show no such reduction in the total potassium content of the soil with increase in depth of sampling. Only a few determinations were made, but these results, listed in table 5, indicate that this fraction cannot account for the decreases observed with the water-soluble and replaceable potassium.

It appears as if these soils, though markedly different in their water-soluble and replaceable potassium content at the surface, might all be found to be very similar at some depth beyond 4 feet. A few samples have been taken to a depth of 8 feet and suggest that such a minimum concentration is attained and maintained in the deeper samples (table 6).

Although sodium and potassium are closely allied chemically it has been generally established that potassium is more readily retained by the soil. Only seven of the soils have been analyzed for sodium and the analyses show no evidence of an accumulation of this particular cation at the surface. The exchangeable sodium either remains fairly constant or increases in the deeper samples. Prune trees growing in Colusa 2 orchard, which is particularly high

TABLE 4  
*Replaceable and active potash content of dry soil in relation to the depth of sampling*

ORCHARD	DEPTH	POTASSIUM CONTENT		
		Replaceable	Active	Difference
	<i>feet</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>
Chico 1.....	1	191	159	-32
	2	89	96	+7
	3	81	74	-7
	4	85	65	-20
Chico 1 . . . . .	1	434	468	+34
	2	240	264	+24
	3	180	190	+10
	4	154	152	-2
Grimes 1 . . . . .	1	1,242	1,455	+213
	2	1,185	1,400	+215
	3	1,027	1,133	+106
	4	489	564	+75
Gilroy 1 . . . . .	1	461	500	+39
	2	270	304	+34
	3	143	160	+17
	4	119	.. .	....
Morgan Hill . . . . .	1	383	448	+65
	2	221	250	+29
	3	168	197	+29
	4	142	.....	.....
Paradise Valley 1.....	1	341	302	-39
	2	174	192	+18
	3	175	167	-8
	4	143	151	+8

in sodium, are showing some distress. Alfalfa and grain crops adjacent to this orchard, however, do well.

Analyses were also made on the water-soluble calcium and magnesium content of soil in relation to depth of sampling and the data concerning these two constituents show no definite trend of change in the concentration of either of these two ions with increase in depth.

TABLE 5  
Total potassium content in relation to depth of sampling

ORCHARD	SAMPLE NUMBER	DEPTH	K
		<i>feet</i>	<i>per cent</i>
Grimes 1 (Faxon).....	390	1	1 47
	391	2	1 36
	392	3	1 35
	393	4	1 23
Chico 2 (Canfield).....	436	1	0 92
	437	2	0 82
	438	3	0 72
	439	4	0 79
Chico 2 (Canfield) .....	472	1	1 02
	473	2	0 93
	474	3	0 96
	475	4	0 95
Gilroy 1 (Barbari) .....	197	1	1 92
	198	2	2 02
	199	3	1 83
	200	4	1 79
Morgan Hill 1 (Hansen) . . . . .	201	1	2 10
	202	2	2 16
	203	3	1 96
	204	4	1 82
Paradise Valley 1 (Ward) .....	205	1	0 89
	206	2	0 78
	207	3	0 89
	208	4	1 04

TABLE 6  
Replaceable potassium content of dry soil

ORCHARD	DEPTH OF SAMPLING							
	First foot	Second foot	Third foot	Fourth foot	Fifth foot	Sixth foot	Seventh foot	Eighth foot
	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
Chico 3 .....	430	240	130	100	90	85	85	80
Chico 4.....	350	130	85	80	70	75	75	70
Chico 4.....	400	155	80	80	85	70	75	60
Chico 4 .....	505	330	235	170	140	115	85	80

Similarly the analyses on the replaceable calcium and magnesium content of soil in relation to depth of sampling<sup>3</sup> show that the replaceable calcium in

<sup>3</sup> No corrections have been made for Ca and Mg that may be dissolved by the ammonium acetate solution.



most of these soils, except those from the Berryessa and Richfield orchards, remains relatively constant with a tendency to be slightly lower in the deeper samples. Replaceable magnesium in these orchard soils, like calcium, shows no distinct trend to increase or decrease. The deeper samples as a rule show a slightly higher magnesium content.

#### DISCUSSION

The distinct reduction in the potassium content of these orchard soils in the deeper samples is so general and consistent as to cause one to speculate on the cause of this concentration gradient. Any solution offered to account for the higher potassium content of the surface soils must also take into consideration its chemically allied cation, sodium, whose concentration either remains fairly constant or tends to increase in the lower depths. It must also be in accord with the data obtained concerning the divalent cations calcium and magnesium whose concentrations in the various depths studied remain relatively uniform. Undoubtedly many factors are involved and further analyses of these soils would be helpful in formulating a theory, but such are not within the scope of the present investigation.

A partial explanation that does not seem to be contradictory to the present data is the influence of vegetation on the chemical composition of the surface soil. Plant residues contain relatively large amounts of potassium. The computed average composition of 24 varieties of grasses as originally determined by Wolff (6) indicates the predominance of potash. Potassium may be absorbed by plants from the deeper soil layers. Upon the death and decay of these plants, the potassium present in their tissues augments the replaceable potassium in the surface soil.

*Average composition of 24 varieties of grasses (hay and straw)*

	<i>Per cent dry weight</i>
K <sub>2</sub> O... ..	.1 49
Na <sub>2</sub> O. . . . .	0 20
CaO.....	0 90
MgO... ..	0 29

It seems probable that under conditions of limited rainfall such as are experienced in the prune growing sections of California, a potassium gradient could have been established and maintained by native vegetation.

Analyses of soils from dry yards adjacent to some of these orchards seem to confirm this hypothesis. These dry yards are areas in which the fruit is spread on wooden trays to dry and which are generally allowed to grow to native grasses and weeds or often are planted to cereal hays which are cut just prior to the fruit harvest. Growers assured me that no tree crops had ever been planted in these sampled areas nor had any manure ever been applied. Yet these soils show potassium gradients similar to those found in the orchards. The data on these dry yard areas are presented in table 7.

There may be several factors operating to preclude the establishment of like concentration gradients of the other cations. Potassium is a specially favored element in that it is most readily fixed in the soil and may replace calcium and other cations. The ease with which it enters into the replaceable complex may account in some degree for the absence of marked changes in concentration of these other cations with depth of sampling.

The solubility of calcium and magnesium carbonates and other salts containing these cations may be great enough to obscure any gradient in concentration produced by plant residues. Kelly and Arany (2) report the existence of relatively large amounts of Ca and Mg as compounds other than replaceable and carbonate forms soluble in ammonium chloride solution.

TABLE 7  
*Potassium content of dry soil from dry yard areas adjacent to prune orchards*

DRY YARD	WATER-SOLUBLE K (1-1)				REPLACEABLE K			
	First foot	Second foot	Third foot	Fourth foot	First foot	Second foot	Third foot	Fourth foot
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Saratoga 2 (Payne) .. . . .	12 8	4 6	1 9	2 1	458	261	131	143
Chico 5 (Stiles) .. . . .	10 5	3 1	2 5	1 8	572	320	274	157

#### SIGNIFICANCE IN SOIL SAMPLING

Recent studies indicate that the fraction of the soil potassium that is replaced by the ammonium ion is available to plants (5, 7). In most soils this fraction is a measure of the soil productivity as regards potassium. Professor Hoagland of the plant nutrition division has, however, called my attention to a soil which is low in replaceable potassium and yet is able to maintain an adequate supply of potassium from other types of minerals present in this particular soil. Although the decreases in available potash content of soils with increase in depth may not be of importance to annual crops, they may have some significance in the study of the nutritional requirements of deciduous fruit trees. Certain experimental field studies in which potash was distributed deep in the soil (3) in comparison with potash applied on the surface indicate that greater response is obtained with the deeper distribution. With differences existing between the surface foot and those deeper down and with our present lack of knowledge as to the relative importance of the various depths of soil as a source of inorganic nutrients for deciduous fruit trees, it would seem desirable to examine orchard soils more critically than a composite or surface sample will permit.

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# DETERMINATION OF THE RATE OF DECOMPOSITION OF ORGANIC MATTER UNDER FIELD CONDITIONS<sup>1</sup>

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In studying the effects of the decomposition of sugar cane trash on the total and available nitrogen of the soil,<sup>2</sup> it was found that as the carbohydrate fraction of the organic material added disappeared the available nitrogen in the soil tended to increase. Practically all of the soil organic matter is found in the lignin-humus, protein, and carbohydrate fractions, and the total soil organic matter can be estimated from the total organic carbon content (6). This condition makes possible the calculation of the carbohydrate fraction by difference. Since the greater part of sugar cane trash is composed of carbohydrates, it was desirable to develop a more direct method of measuring the rate of the decomposition of the carbohydrate fraction of the trash in the soil.

## EXPERIMENTAL METHODS

A series of closely controlled 1/2,000-acre field plats on Sharkey silty clay loam were treated with sugar cane trash and ammonium sulfate and kept bare of crops. There were four plats under each treatment and the treatments were as follows:

1. Check, no treatment
2. 12 tons cane trash and 80 pounds nitrogen per acre
3. 12 tons cane trash and 40 pounds nitrogen per acre
4. 12 tons cane trash per acre

The first application of trash and nitrogen was made in March, 1930. The second application was made on October 27, 1930, and the data given here are from the second application. The trash was chopped and then the trash and ammonium sulfate were applied to the soil and mixed with it to a depth of 5 inches.

Samples were taken from the first 6 inches. The soil at the first sampling after being treated with trash had a volume weight of 1.21.

Total nitrogen was determined by the Gunning-Hibbard method.

<sup>1</sup> Published with the approval of the director of the Louisiana Agricultural Experiment Station.

<sup>2</sup> STURGIS, M. B. The effect of additions of nitrogen on the decomposition of sugar cane trash under field conditions. Unpublished data Louisiana Agricultural Experiment Station, 1931.

Organic carbon was determined by the dry combustion method.

Ether extract was determined by extracting the dry material in a Soxhlet for 8 hours. Fifty-gram samples of the soil material were used.

Alcohol extract was determined on the material after the ether extraction by treating it in a Soxhlet for 8 hours with 95 per cent alcohol. The alcohol extract was dried and weighed as in an ether extraction.

Lignin and lignin carbon were determined on the material after the ether and alcohol extractions by a combination of the methods of Dore (2) and Schwalbe (4). A sample weight that would give a residue of not more than 0.10 gm. of carbon was taken. Three samples were run. The nitrogen content of the lignin fraction was run and calculated to protein, the ash content was determined, and the lignin was calculated free of protein and ash. In the determination of the carbon content of the lignin fraction two samples were run. The carbon content of one and the nitrogen content of the other were found. Then the carbon content of the lignin was calculated free of the protein carbon.

Reducing sugars were determined on the carbohydrate material put in solution and hydrolyzed in the treatment for the isolation of the lignin fraction. Reducing sugars were also run on the alcoholic extract of fresh trash. All reducing sugars were determined as anhydrous dextrose. By a comparison of the results of the proximate method of analysis used in this work with the results of the more exact methods of Brown and Blouin (1), it will be noted that most of the error in the proximate method falls in the carbohydrate fraction. This is due not so much as might be suspected to the fact that the different sugars have different reducing values and that the non-reducing sugars are not determined, for dextrose makes up more than 60 per cent of the sugars, with xylose, arabinose, levulose, and galactose being represented in correspondingly small amounts as the only other sugars occurring in appreciable quantities. The greatest error introduced into the determination of all the carbohydrates by hydrolysis to reducing sugars is due to either incomplete hydrolysis or decomposition of hydrolyzed products or both. Strong acid and high temperatures will cause decomposition and humification of the hydrolyzed material (5), which will be reflected in low yields of reducing sugars and too high results for lignin or lignin-humus.

The reducing sugars from the hydrolysis of the carbohydrates in trash were determined on the filtrate from the lignin residue. But it was found that for soil material a separate sample had to be taken. The following method gave somewhat low but consistent results:

The soil material remaining after the ether and alcohol extractions was dried and a 20-gm. sample taken. The sample was placed in a 500-cc. glass stoppered Erlenmeyer flask and moistened with 5 cc. of 18 per cent hydrochloric acid. Then 25 cc. of 72 per cent sulfuric acid was pipetted into the flask, care being taken to wash all the soil material to the bottom; the glass stopper was lubricated with the sulfuric acid, placed in the flask, and weighted down against the pressure. The pressure of HCl will be from 20 to 30 mm. above atmospheric pressure. The flask was rotated to mix completely the acids and sample and quickly placed in a water bath at 20°C. After 24 hours, the contents of the flask were diluted with 300 cc. of water and boiled under a reflux condenser for 1 hour. The solution was filtered through a Gooch

crucible and the lignin-humus residue washed free of acids. The iron, aluminum, and manganese were precipitated from the filtrate with sodium carbonate and sodium hydroxide. Two precipitations were necessary. Sodium carbonate was added to the solution until precipitation began, then a 10 per cent solution of sodium hydroxide was added to bring the solution up to pH 6.0. The mixture was made up to 400 cc. and filtered through a Büchner funnel provided with a filter paper and asbestos mat. Sodium hydroxide solution was then added to the filtrate until the mixture was very slightly alkaline, pH 9.0. The volume of the alkaline solution used in the second precipitation was noted and added to the original volume of 400 cc. in the calculation. A second filtration was made using a new filter paper and mat. Reducing sugars were then determined as anhydrous dextrose on the clear, slightly alkaline filtrate by the method of Lane and Eynon (3) for the determination of reducing sugars by means of Fehling's solution with methylene blue as an internal indicator. Very low concentrations required that the method be modified for the use of 2 cc. of Fehling's solution and that a table of factors and dextrose values be made for the conditions of the determination.

The soil used in this experiment did not contain sufficient higher oxides of manganese to liberate chlorine from the hydrochloric acid, but the hydrolysis of carbohydrates in soils that contain higher oxides which will liberate chlorine from hydrochloric acid can be better accomplished by the use of sulfuric acid only.

A test of the method on 1-gm. samples of material which contained 99.3 per cent cellulose showed that it was possible to recover 97.8 per cent of the cellulose as dextrose. There was a residue of 2.4 per cent of the sample of which a part was degraded and a part not hydrolyzed. In studying the hydrolysis of the same material in the presence of soil, it was possible to recover only 91.1 per cent of the cellulose as dextrose.

The percentage of the added carbohydrates that had decomposed was calculated from the difference between the reducing sugars present in the soil at a given time and the sum of the reducing sugars in the soil at the application of the trash and the expected increase from the addition of the trash, the expected increase from the trash being considered as 100 per cent.

## RESULTS

The results given in tables 1 and 3 following the various treatments are the averages from samples taken from the four plats under each treatment.

Data in table 3 indicate that there was a very appreciable disappearance of the carbohydrate fraction as reducing sugars within the first month following

TABLE 1  
*Organic matter in the soil before application of the cane trash and nitrogen*  
Results are on the basis of dry soil

DATE	TREAT- MENT	TOTAL N	ORGANIC C	N C	LIGNIN- HUMUS C	REDUCING SUGARS
		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
October 27, 1930. ....	1	0.1053	1.074	1:10.2	0.493	0.308
	2	0.1118	1.165	1:10.4	0.535	0.352
	3	0.1110	1.167	1:10.5	0.538	0.353
	4	0.1093	1.139	1:10.4	0.524	0.341

TABLE 2

*Composition of the cane trash applied on October 27\**

Moisture is on the basis of moist field weight. Other determinations are on the dry basis.

	PER CENT
Moisture.. . . . .	70.55
Ash.. . . . .	9.92
Total N.. . . . .	0.82
Total C.. . . . .	41.95
Ether extract . . . . .	2.57
Alcohol extract (less reducing sugars in alcohol extract) . . . . .	5.10
Lignin (62.8 per cent C).....	13.23
Total reducing sugars (as anhydrous glucose) . . . . .	63.45

\* Had it been possible to sample the soil immediately after applying the trash the percentages of nitrogen, carbon, and reducing sugars in the soil would have increased 0.0035, 0.180, and 0.272 respectively.

TABLE 3

*Organic matter in the soil after the application of the cane trash and nitrogen*

Results are on the basis of dry soil

DATE	TREAT- MENT	TOTAL N	ORGANIC C	N. C	ETHER AND AL- COHOL EX- TRACT	LIGNIN HUMUS C	REDUC- ING SUGARS	ADDED CAR- BOHY- DRATES DECOM- POSED
		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
November 25, 1930.....	1	0 1054	1 062	1:10 1	0 037	0 488	0 307	.
	2	0 1200	1 384	1:11 5	0 050	0 613	0 513	40 7
	3	0.1157	1 339	1:11 6	0 056	0 626	0 526	36 4
	4	0 1114	1 302	1:11 7	0 054	0 612	0 512	37 1
January 22, 1931.....	1	0 1060	1 058	1:10 0	0 037	0 483	0 306	.....
	2	0 1188	1 311	1:11 0	0 050	0 575	0 465	57 7
	3	0 1162	1 304	1:11 2	0 052	0.597	0.491	49 2
	4	0.1126	1.306	1:11 6	0 046	0.612	0 508	38 6
March 11, 1931...	1	0.1060	1 063	1:10 0	0 042	0 490	0 278	...
	2	0.1155	1 229	1:10 6	0 052	0 547	0 401	81 2
	3	0 1157	1 271	1:11.0	0 046	0.574	0.413	77 9
	4	0 1142	1 294	1:11 3	0 051	0 597	0.453	58.8
June 12, 1931. . . . .	1	0.1040	1.044	1:10 0	0.041	0 464	0 275	.....
	2	0 1173	1.254	1:10.7	0.046	0.579	0 371	92.3
	3	0.1163	1 233	1:10 6	0 050	0 581	0 388	87.1
	4	0 1140	1.226	1:10 8	0.041	0 572	0 402	77.6
August 1, 1931 .....	1	0.1049	1 048	1:10.0	0.039	0.460	0.271	.....
	2	0.1159	1 180	1:10 2	0 050	0 501	0.324	109.6
	3	0.1129	1.164	1:10.3	0.048	0.521	0 324	110.7
	4	0.1119	1.153	1:10 3	0 044	0.520	0.338	101.1

the application of the trash and ammonium sulfate. The results from the first sampling are not entirely consistent and the discrepancies can be partly explained by the fact that it is very difficult to get a truly representative sample before the trash is largely decomposed. Later results show that the carbohydrate fraction decomposes much more readily than the lignin-humus and protein fractions and that the addition of nitrogen with trash increases the rate of the decomposition of the carbohydrates. A slightly greater amount of carbohydrate than was added through the trash disappeared from the plats that received trash only in the course of the experiment. This extra decomposition of the carbohydrates already in the soil before the application of the trash is also indicated from the fact that the nitrogen-carbon ratio for treatment 4 was lower at the end of the experiment than at the time the trash was applied (table 1). Decomposition of the carbohydrates and the narrowing of the carbon-nitrogen ratio are in good agreement with each other and show that all the trash applied had decomposed to a greater degree or to a more narrow carbon-nitrogen ratio than existed in the soil when the trash was applied.

The lignin-humus fraction of the trash decomposed slowly but it did not accumulate as did the protein fraction.

The protein or nitrogen fraction was the most resistant part of the soil organic matter and because of the synthetic processes of the microorganisms involved in the decomposition of a material which contained a small amount of nitrogen and a great amount of carbohydrates, the nitrogen fraction accumulated in the soil.

#### CONCLUSIONS

Since the various fractions of organic materials decompose at different rates in the soil, it is important to be able to determine by as direct method as possible changes in the amounts of the major fractions of the soil organic matter.

A method for the determination of the carbohydrates in the soil as reducing sugars has been developed and found to give good results in measuring the rate of the decomposition of the carbohydrate fraction of sugar cane trash in the soil.

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# THE SOIL AS A HABITAT FOR GROWTH OF GREEN ALGAE

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It has been known for a long time that the soil harbors blue-green and green algae. Until recently, however, it has been assumed that the algae were chance invaders, and that they multiplied only under conditions of extreme moisture and only on the surface. The early work of Esmark (4), Peterson (9), and Robbins (10) demonstrated a large number of species in soil. Later work by Bristol Roach (1, 3) and by Moore and associates (7, 8) in normal soil and in soil dried for a half century also showed that algae were found in deep as well as surface soil in large numbers. Bristol Roach found in many soils more than 100,000 cells per gram (2, 3).

The fact that algae are found in soil deep enough to prevent any possible nutrition by photosynthesis should not cause surprise, inasmuch as it has been known that many species of green algae are able to grow saprophytically in complete darkness for years without a diminution of their activities, and with the production of abundant chlorophyll, provided sufficient energy in the form of available organic nutrients is present.

That algae actually exist in the soil in an active non-resting state was first demonstrated by Bristol Roach (2, 3) using a modification of McLennan's (6) ingenious technique for "counting" active fungi in the soil. Bristol Roach found that rapid drying of the soil destroyed the vegetative cells of certain species of green algae without destroying the resting cells. Her method (3)

"consists in counting the total number of individuals in one part of a well-mixed freshly gathered soil sample by means of a cultural dilution method, and in counting the number of resting or resistant forms in a second part of the same sample after it has been subjected to rapid desiccation by means of a stream of dried sterile air at laboratory temperature, the vessel containing the soil being immersed in a water bath at 35°C. so that the conditions should approximate those of a dry wind on a hot day. A comparison of the two counts gives some idea of the proportion of vegetative cells to resting cells. It has been found that the different species do not all react in the same manner to this treatment but that their resistance to desiccation is very varied: diatoms appear to be entirely killed off and are therefore presumably all present in the soil in the vegetative condition; blue-green algae, except for one small sheathless species of *Oscillatoria* which was completely killed off, appear to be unaffected by the treatment, while the unicellular green forms reacted quite specifically. In one particular sample, *Pleurococcus vulgaris* Menegh, the most resistant species, was reduced only to rather more than one-third, while *Chlorococcum humicola* was reduced to one-twelfth of its numbers.

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In *Chlorella* sp. and *Heterococcus viridis* more than ninety-nine out of every hundred cells were killed off, and in *Bumilleria exilis* a few cells only survived. In fact, of a total algal population of about eighty thousand individuals per gram of soil, only about nine thousand survived after desiccation. This definite proof that the soil algae are actually present in large numbers in the vegetative condition raises their status as members of the soil population to a position not far short in importance of that of the other groups of soil organisms, the numbers of individuals in the Rothamsted soil being quite comparable with those of the protozoa."

Definite information that algae actually *multiply* in soil, however, has not been encountered by the author. It was to supply this missing link in the chain of evidence that algae lead an active life in normal soils that the following experiments were carried out.

#### EXPERIMENTAL

Samples of peat (Niedermoor), garden loam, and sandy field soil were taken from the field when in a moist but not wet condition. Unfortunately no moisture equivalent determinations were made but it may be stated that the soil was friable and appeared to contain free water to the extent of somewhere from one-half to two-thirds of the water holding capacity. This soil was weighed in 100-gm. amounts and added to 250-cc. Erlenmeyer flasks, which were then plugged with cotton wool and immersed in a water bath for 90 minutes at 85°C. They were removed and weighed and left until they had lost in weight about 10 gm. Heating the soil thus does not kill all the bacteria. It greatly reduces their numbers but the total numbers again rise to very high levels relatively soon. The fungi, algae, and protozoa are all, or nearly all, eliminated, and their bodies undergo a bacterial decomposition similar to what happens after moistening soil following air drying. Carbon dioxide and ammonia production increases and ammonia rather than nitrates, accumulates in the soil but it is well known that most algae are able to use nitrogen in either form.

At this time dilute suspensions of two pure cultures of unicellular algae<sup>2</sup> isolated from soil (11) were prepared, and 10 cc. of each were added to duplicate flasks of each of the three samples of soil, making 12 flasks in all. An equal number of flasks of soil were left uninoculated, and 10 cc. of sterile water was added. The flasks were all wrapped in heavy paper and placed in tin cans in which a few holes plugged with cotton wool, had been prepared for ventilation. All of the cans were placed in a dark cupboard and left for 6 and 8 months. Once a month sterile distilled water was added to make up for loss by evaporation. Tubes containing inorganic media inoculated with algae failed to show growth, indicating that the darkness was complete. After the long incubation the soil was removed, carefully mixed, and the numbers obtained by the dilution method using the tables of Halvorson (5) and the liquid medium of Beijerinck (11). At the time of inoculation the algae were counted

<sup>2</sup> The algae were identified as *Chlorella* sp. and as probably *Scenedesmus costulatus* Chodat var. *chlorelloides* Bristol Roach.

TABLE 1

*Number of viable units of Chlorella sp. per gram of partially sterilized soil at time of inoculation and after 6 months' incubation*

NUMBER OF POSITIVE TUBES PER DILUTION				MOST PROBABLE NUMBER OF VIABLE UNITS PER GRAM OF SOIL
1:10	1:100	1:1,000	1:10,000	
At inoculation				
4	0	0	..	3.8
2	1	0	..	
Sandy soil. After incubation				
10	8	1	..	125
10	7	0	..	
Peat. After incubation				
..	10	4	0	642
..	10	6	0	
Loam. After incubation				
..	10	9	2	2,280
..	10	9	2	

TABLE 2

*Number of viable units of Scenedesmus costulatus var. chlorellioides (?) per gram of partially sterilized soil at time of inoculation and after 8 months incubation*

NUMBER OF POSITIVE TUBES PER DILUTION				MOST PROBABLE NUMBER OF VIABLE UNITS PER GRAM OF SOIL
1:10	1:100	1:1,000	1:10,000	
At inoculation				
7	2	0	..	13.3
7	1	1	..	
Sandy soil. After incubation				
10	7	1	..	97.1
10	6	0	..	
Peat. After incubation				
10	10	3	..	565
10	10	5	..	
Loam. After incubation				
..	10	8	1	1,330
..	10	7	1	

in a similar manner in two flasks of soil prepared for the purpose. The first dilution was made by adding water to 10 gm. of moist soil to the 100-cc. mark. After that, 10 cc. of each dilution was added to 90 cc. of sterile inorganic medium, and 1 cc. of each decimal dilution added to Beijerinck's medium in replicates of 10 and incubated for 8 weeks in a suitable light. The estimates of numbers of viable algae were made in the usual manner by using Halvorson's tables, tubes which showed any green growth being counted as positive. None of the tubes inoculated with the soil to which no algae had been added showed growth. The results for the others are shown in tables 1 and 2.

#### SUMMARY

The results of the experiments show that algae inoculated in soil partially sterilized by heat are able to increase as much as 500 fold when incubated at room temperatures in total darkness for long periods of time. They offer added evidence that soil is a habitat for certain green algae and that the algae multiply in soil under conditions of darkness and of moderate moisture.

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# NOTES ON THE ASSOCIATION OF MICROÖRGANISMS AND ROOTS<sup>1</sup>

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Greater numbers of microörganisms in the immediate neighborhood of plant roots than outside the root-zone or rhizosphere have been reported by various workers. Some "influence" of the roots which brought about these increases was assumed, but not analyzed. The samples from which cultures were made were carefully collected as "representative" of the area to the depth designated, then homogenized, and sieved to insure the presence of of earthy materials only. Smith and Humfeld<sup>2</sup> before the Society of American Bacteriologists in December, 1929, reported experiments which pointed to such localization of bacteriological activities upon organic masses in soil as to lead to the recommendation that further studies be directed more to the determination of the extent and nature of these localized activities than to further attempts to define population levels by dilution cultures from aliquots drawn from homogenized samples representing great areas and vast masses of soil.

Starkey followed the earlier practices in parts I, II, and III (2, 4, 5) of his studies of the micropopulation of the rhizosphere, but has offered intensive studies of the numbers of microörganisms on the roots themselves in part IV (6). His survey of the whole literature of the field makes a general discussion of the historical aspects of the subject unnecessary.

In his recent paper (6) Starkey took the usual type of samples with elaborate care at three different distances from the main root of the plant; the outermost sample was planned to be clearly outside the zone of influence; the second was taken at an intermediate distance; the third close to, but not in contact with the main center of the root system. The studies included total colony counts on albumin agar and differential counts for fungi, actinomycetes, mucoid colonies, and radiobacter. The plants selected included two series of three species each: (a) second-year growth of the biennials, table beets, mangel beets, and sweet clover, and (b) fresh plantings of bush beans, mangel beets, and corn. His fourth sample consisted of scrapings of the roots themselves, which necessarily included adherent soil particles. As in previous reports, the numbers of microörganisms were greater as the samples entered the root zones and approached the main roots of the plants but reached totals which averaged 45.3 times (6, p. 375) as many bacteria in all of the samples of root scraping as in all of the samples of nearby soil. In the legumes this ratio was 113.3, but in the non-legumes it fell to 11.3. These results indicate clearly that increased numbers of microörganisms in the root zone are due to organisms actually in or on the roots themselves.

Independently following the same general lines, several series of samples were studied in the laboratories of the Bureau of Chemistry and Soils during the past year, which give results in general harmony with those of Starkey and extend the field of investigation in certain directions.

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This paper was presented at the annual meeting of the American Society of Bacteriologists, at Baltimore, Maryland, December 30, 1931.

<sup>2</sup> Abs. in *Jour. Bact.* 19 (1): 43-44. 1930.

## STUDIES OF RYE, VETCH, AND ALFALFA ROOTS

The procedure used in sampling for the comparative study of the populations in soil and on the roots of rye, vetch, and alfalfa during the early spring of 1931 follows. A typical healthy plant was dug up, with care to disturb the root system as little as possible. The adhering soil was shaken off and labelled "soil adjacent to roots." The fibrous roots were cut from the main root system for a separate sample. This included the soil particles which adhered so closely that vigorous shaking did not dislodge them. A sample was taken from fallow soil outside of the root zone.

TABLE 1  
*Distribution of microorganisms in relation to roots of plants in different soils*

TYPE OF SOIL	KIND OF MATERIAL	CROP	pH	NUMBER OF FUNGI PER GRAM	NUMBER OF ACTINO-MYCES PER GRAM	NUMBER IN TOTAL PLATE COUNT PER GRAM (BACTERIA)
Keyport clay loam... ..	Soil	.....	5.7	130,000	4,030,000	30,000,000
	Soil adjacent to roots	Alfalfa	5.8	251,000	4,010,000	98,350,000
	Fibrous roots	Alfalfa	6.2	620,000	13,750,000	423,000,000
Keyport clay loam.....	Soil	.....	6.1	90,000	715,000	99,500,000
	Soil adjacent to roots	Rye	6.9	121,000	890,000	92,500,000
	Fibrous roots	Rye	7.2	575,000	2,600,000	506,000,000
Leonardtown clay loam....	Soil	... .	6.1	287,000	2,500,000	24,050,000
	Soil adjacent to roots	Rye	6.8	307,000	1,250,000	47,050,000
	Fibrous roots	Rye	7.6	1,430,000	...	527,500,000
Leonardtown clay loam....	Soil	.... .	6.8	261,000	1,325,000	42,050,000
	Soil adjacent to roots	Vetch	7.0	260,000	.	162,500,000
	Fibrous roots	Vetch	7.0	1,340,000	1,550,000	495,000,000
Collington fine sandy loam.	Soil	... .	6.4	22,000	1,700,000	18,000,000
	Soil adjacent to roots	Rye	6.4	32,000	1,950,000	21,650,000
	Fibrous roots	Rye	7.1	330,000	3,350,000	292,000,000

Ten grams of each sample were weighed out, triturated in a mortar, and suspended in 250 cc. of sterile water. Dilutions on the basis of 10 cc. of inoculum to 90 cc. of sterile water were used, and the series carried far enough to insure a suspension which would give a favorable number of colonies for counting. Five cubic centimeters of the suspension were placed in a sterile petri dish and the desired medium was added.

The media used [following Smith and Humfeld (2)] were acid dextrose soil extract agar for the fungi, glycerine nitrate soil extract agar for the actinomycetes, and soil extract agar for the total plate counts of bacteria.

The plates for fungous colonies were incubated for 3 days, those for actinomycetes 10 to 14 days, and the total count plates for 21 days.

Table 1 gives a summary of the results obtained in this first series of samples.

The soils were Keyport clay loam, Leonardtown clay loam, and Collington fine sandy loam. The pH of the soils varied from 5.7 to 6.8, and the pH of the roots was from 6.2 to 7.6. In each case the roots were somewhat less acid than the soil.

The number of fungi on the fibrous roots was considerably higher than the number in the soil. The actinomycetes in most cases show an increase on the roots. However, difficulty was experienced in obtaining an accurate count because fungi and bacterial spreaders grow rapidly on this medium and often mask or inhibit actinomycetes so that an accurate count was not always possible. The total plate counts, which were mostly bacteria, were always very high from the fibrous root material, varying from 292 million for the rye roots from the sandy soil, to 527 million for the rye roots from the clay loam. The number in the soil was the usual number, varying from 18 million for the sandy soil to 99 million for the Keyport clay loam.

The number of microörganisms in the rhizosphere, that is, the soil adjacent to the roots, was usually higher than the number in the soil outside the root zone.

#### CORN ROOTS IN SEVEN SOILS

In connection with another experiment in progress in the greenhouse, it was possible to obtain roots of corn which had been grown under identical conditions, except that the soils varied. The corn at time of sampling was about 8 weeks old and about 2 feet high. The plots had grown a number of successive crops of corn and each crop had received an application of phosphate and potash.

The soils were Caribou loam, pH 4.5; Collington fine sandy loam, pH 4.8; Keyport clay loam, pH 5.9; Carrington loam, pH 7.; Houston clay loam, pH 7.8; a silt loam from Colorado, pH 7.9; and an "alkali" soil from Colorado, pH 8.1. The same media were used for fungi, actinomycetes, and total plate counts as in the previous experiments. The results are summarized in table 2.

In the acid soils and in the neutral soil the roots were less acid than the soil; in the alkaline soils the roots were less alkaline than the soil. The colony counts of fungi, actinomycetes, and bacteria varied as in the previous series of samples. It was noted that the number of fungi on the roots of corn was greatest in the most acid and in the most alkaline soil. The number of actinomycetes did not show any increase and the number of bacteria was greater on the roots grown in soil slightly acid or neutral.

Group identification of the fungi showed that species of *Trichoderma* predominated in the roots in acid to neutral soils, whereas in the three alkaline soils biverticillate *Penicillia* (the *P. luteum* group) predominated.



TABLE 2

*Distribution of microorganisms in relation to corn roots in soils of different acidity*

TYPE OF SOIL	KIND OF MATERIAL	pH	NUMBER OF FUNGI PER GRAM	NUMBER OF ACTINOMYCETES PER GRAM	NUMBER OF TOTAL PLATE COUNTS (BACTERIA)
Caribou loam.....	Soil	4.5	145,000	425,000	24,500,000
	Soil adjacent to roots	4.8	230,000	.....	19,000,000
	Fibrous roots	6.1	4,100,000	.....	43,350,000
Collington fine sandy loam.....	Soil	4.8	100,000	1,100,000	5,500,000
	Soil adjacent to roots	5.2	800,000	.....	26,000,000
	Fibrous roots	5.6	7,000,000	.....	136,000,000
Keyport clay loam .	Soil	5.9	105,000	465,000	33,300,000
	Soil adjacent to roots	6.1	200,000	.....	53,150,000
	Fibrous roots	6.7	555,000	1,900,000	260,000,000
Carrington loam ...	Soil	7.0	141,000	5,000,000	35,000,000
	Soil adjacent to roots	7.0	70,000	2,225,000	38,000,000
	Fibrous roots	7.6	700,000	5,000,000	270,000,000
Houston clay.....	Soil	7.8	155,000	955,000	25,450,000
	Soil adjacent to roots	8.0	.....	.....	.....
	Fibrous roots	7.5	300,000	1,000,000	82,000,000
Silt loam Colorado..	Soil	7.9	205,000	1,020,000	41,000,000
	Soil adjacent to roots	7.7	162,000	490,000	48,500,000
	Fibrous roots	7.5	352,000	1,400,000	60,000,000
"Alkali" soil Colorado.	Soil	8.1	100,000	930,000	17,750,000
	Soil adjacent to roots	8.1	185,000	495,000	25,650,000
	Fibrous roots	7.5	1,315,000	450,000	138,000,000

## EFFECT OF TOBACCO ROOT ROT ON THE NUMBERS OF MICROORGANISMS

As plants of tobacco, both healthy and infected with the tobacco root rot (*Thielavia*) were available, it was thought that some interesting information might be gained by plating out the organisms on the roots of these plants. Three different samples were obtained: (a) from a highly resistant Burley strain grown on badly infected soil; (b) from a susceptible strain grown on soil free from infection as far as known; (c) from a susceptible strain grown on badly infected soil, in fact, grown adjacent to the resistant strain.

The results obtained are shown in table 3. It will be seen at a glance that large differences exist. Total plate counts gave 136 millions on healthy, uninfected plants grown on root-rot free soil, 223 millions on the resistant strain in infected soil and 1,620 millions on the susceptible strain on infected soil. The results on the other media were proportionate in a general way. It was found that radiobacter was practically absent on the roots of plants in

TABLE 3  
*Numbers of microorganisms on washed roots on tobacco plated on different media*

KIND OF ORGANISMS	MEDIUM	FIBROUS ROOTS RESISTANT— FECTED SOIL BURLY VARIETY ON IN-	LARGER ROOTS RESISTANT— FECTED SOIL BURLY VARIETY ON IN-	FIBROUS ROOTS FROM UNIN- FECTED SOIL	LARGER ROOTS ON UNIN- FECTED SOIL	FIBROUS ROOTS BADLY IN- FECTED	NUMBERS ON LARGER ROOTS
Total colony count.....	Soil extract agar	233,000,000	69,000,000	136,000,000	27,000,000	1,620,000,000	181,000,000
Fungi.....	Acid dextrose nitrate agar	45,000	20,000	62,000	142,000	182,000	20,000
Actinomycetes.....	Glycerine nitrate soil ex- tract agar	4,500,000	2,500,000	2,300,000	550,000	3,200,000	1,000,000
Mucoid colony count.....	Glycerine nitrate soil ex- tract agar	3,000,000	3,700,000	1,800,000	.....	35,000,000	4,700,000
Total colony count.....	Glycerine nitrate soil ex- tract agar	115,000,000	74,000,000	40,000,000	13,200,000	1,500,000,000	102,000,000
Mucoid colony count.....	Crystal violet glycerine nitrate soil extract agar	850,000	600,000	140,000	250,000	50,000,000	200,000
Total colony count.....	Crystal violet glycerine ni- trate soil extract agar	41,750,000	55,000,000	1,140,000	625,000	154,000,000	4,400,000
Mucoid colony count.....	Mannite agar	5,800,000	.....	1,000,000	500,000	50,000,000	4,000,000
Total colony count.....	Mannite agar	212,000,000	125,000,000	29,600,000	7,100,000	1,400,000,000	112,000,000
Total colony count.....	Albumin agar	245,000,000	200,000	50,700,000	17,800,000	640,000,000	105,000,000
Total colony count.....	Dextrose agar	78,000,000	40,700,000	19,600,000	20,000,000	865,000,000	130,000,000
Total colony count.....	Starch agar	225,000,000	168,000,000	37,500,000	20,500,000	1,620,000,000	122,000,000
Total colony count.....	Peptone agar	230,000,000	135,000,000	10,500,000	20,000,000	890,000,000	165,000,000

uninfected soil, whereas 50 million per gram were present on the susceptible infected roots. The total number of colonies on mannite agar and on starch agar was especially high. No doubt, the root-rot organism weakened the resistance of the root cells against invasion by other organisms and as the root cells died the number of microorganisms present increased tremendously.

In the preliminary experiments reported in table 1, three soils were studied: Keyport clay loam with pH at 5.7 and 6.1, Leonardtown clay loam at pH 6.1 and 6.8, and Collington fine sandy loam at pH 6.4; three crops, rye, vetch, and alfalfa were grown upon them. In each case, the root samples with such soil particles as adhered to them were somewhat less acid than the soil in which they had grown. Of the colony counts of microorganisms the actinomycetes showed less differences than the bacteria or fungi. Their numbers from the roots varied from slight increases or decreases to about 4 times those found in the surrounding soil; the fungous colonies were 5 to 15 times as numerous on the roots, and the bacteria 5 to 20 times as numerous on the roots as in the soil. Although the procedures were so different that the figures are not directly comparable, these results may be regarded as fully in harmony with those of Starkey (6).

These results suggested the utilization of the corn already growing in the greenhouse upon seven plots of soil differing in origin, texture, and acidity but under comparable conditions of temperature, illumination, and watering. These plots varied from fine sandy loam to heavy clay and from pH 4.5 to pH 8.1. Here again, with a single crop, the root samples from the corn grown upon the acid soils were less acid than the soil and those grown upon alkaline soils were less alkaline. There was a clear tendency of the root systems to maintain a reaction approaching the neutrality zone between pH 6 and pH 7.5 rather than to show the acidity or alkalinity of the soil.

The microorganisms outside of the root tissues inhabit a sort of horizon zone between the root juices with one reaction and the contrasting solution of the surrounding soil. Comparison of the colony counts of fungi shows 4,100,000 to the gram on the roots from Caribou at pH 4.5; 7,000,000 from Collington, a loose textured fine sandy loam, at pH 4.8; 700,000 from Carrington of texture similar to Caribou, but at pH 7.0, and up to 1,315,000 again from roots grown in the "alkali" sample from Colorado at pH 8.1, which also is a fairly open textured soil. The high figures for bacteria were from roots grown in Keyport at pH 6.7 which gave 260,000,000, and in Carrington (pH 7.0), 270,000,000 to the gram of roots. These figures harmonize in a general way with the experience that fungous colonies are reduced where conditions are favorable to excessive growth of bacteria and vice versa, but the data are inadequate more than to show the trend.

The figures already discussed showed so definite a relation between the roots and the microorganisms that another series of cultures was made from healthy and infected tobacco roots. For this purpose tobacco plants already full grown under these contrasting conditions were available. Tobacco roots

present the opportunity to obtain samples of fibrous roots and scrapings from large roots. The three samples show the conditions produced about resistant roots in infected soil, susceptible roots in uninfected soil, and badly infected roots.

The total colony counts on soil extract agar were 27 millions per gram on healthy roots in uninfected soil; 233 millions on roots of a variety of tobacco resistant to the disease in infected soil; and 1,600 millions on the roots of tobacco of a variety not resistant in infected soil. The detailed figures in table 3 give some picture of the great numbers of organisms and the varieties of activity presented in connection with root diseases.

Colony counts of molds such as these reported here have a purely arbitrary value. Spores scarcely occur in the deeper areas about the root system. The colonies found represent the fragments of mycelium resulting from the grinding of the sample. When that grinding is all done by the same person with a reasonably standardized procedure, the results at least indicate the relative activities of the species viable on the media used. It is interesting to find that corn roots on acid soil are accompanied by abundant growth of *Trichoderma* which is largely absent on the sample from alkaline soil but replaced by the "biverticillate" *Penicillia*.

In addition to the cultures, microscopical studies of fresh roots were made for supplementary information especially as to the molds and their distribution. Mold hyphae are usually difficult to find in mounts made directly from earthy masses, as noted by Conn in his studies of microscopic counting methods, and by others. Upon and in fibrous roots, however, mold hyphae are nearly always evident. For example, one rootlet  $200\mu$  in diameter showed five large adherent brown hyphae of a single type of mold almost equally spaced for a considerable distance. These hyphae showed various anastomoses between those on the side of the rootlet seen with the microscope. Other fungous hyphae were present in the same field. On other rootlets penetration of superficial cells by mold hyphae was seen. Among root hairs, the actual invasion of the lumen of the hair by hyphae was not uncommon. As these are not isolated observations, it is concluded that mold growth microscopically seen in contact with and often inside the cells of the root cortex is common enough to justify the colony counts observed in culture.

Incidentally, myriads of bacteria appeared in the microscopic fields examined for molds although there was no further attempt to follow their distribution with the microscope.

#### SUMMARY

The authors report results in harmony with those of Starkey in indicating that (a) there are increased numbers of microörganisms in samples of soil taken in proximity to plant roots; (b) that these numbers are many times greater when the roots themselves are examined.

The soil samples studied showed acidities expressed as pH 4.8 to pH 18. and from clay loam to sandy loam.

The samples of fibrous roots with adherent soil particles gave reactions less acid than the acid soil and less alkaline than the alkaline soils, the figures ranged from pH 5.6 in one sample of roots from Collington loam at pH 4.8 to roots at pH 7.6 on Carrington loam at pH 7.0 on the one side; and on the other, roots at pH 7.5 on an "alkali" soil from Colorado testing pH 8.1. The high bacterial counts obtained are characteristic of situations which approach the zone around neutrality, i.e., pH 6 to 7.5.

Mold cultures from corn roots in acid soil produced colonies predominantly *Trichoderma*; corn roots from alkaline soil seemed to be associated predominantly with *Penicillia* of the biverticillate series (*P. luteum* and its allies).

High colony counts of fungi occurred on the very acid soils and the very alkaline samples.

The data presented justify the conclusion that corn roots penetrating through masses of soil of either strongly acid or strongly alkaline reaction, still maintain their own reaction in or near the general zone between pH 6 and pH 7.5 in which bacteriological activities are much more intense than under more acid or alkaline conditions. In doing this they create about them a very narrow zone favorable to bacterial and mold activity.

Greatly increased numbers were shown upon samples of roots known to be infected by root rot or to have grown in heavily infected soil.

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# INFLUENCE OF CONTINUOUS AERATION UPON THE GROWTH OF TOMATO PLANTS IN SOLUTION CULTURES<sup>1</sup>

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In the course of an experiment in which it was necessary to grow tomato plants in solution cultures it was observed that the roots of the plants did not grow down through the solution to the bottom of the culture jars. Instead, the roots were greatly branched and thickly packed at the surface of the solution in the necks of the jars. Since it was desirable to prevent this packing of the roots in the jars, and since the crowding at the surface of the solution suggested a lack of oxygen in the solution, the following experiment was carried out to show the effect of vigorous and continuous aeration of the culture solution upon the growth of tomato plants.

No attempt was made to study the oxygen content of the solution. Allison and Shive (2) have previously shown that aeration of solution cultures which maintained the maximum supply of dissolved oxygen in the culture solutions renewed intermittently had no apparent influence upon the growth of soybean tops, but it produced considerable increase in root development. When the solution was continuously renewed, however, they found that aeration produced a marked increase in the growth of both tops and roots of soybeans.

## CULTURE METHODS

Tomato seedlings of the Marglobe variety were germinated in sand and were transferred to the culture solution when they were about 6 days old. The seedlings were suspended in cork stoppers in the 2-quart fruit jars used as culture vessels, in a manner similar to that described by Tottingham (5). Three plants were used in each culture vessel. Plants grown during an experimental period of 81 days were started in the 2-quart jars and single plants were removed from these at the age of 27 days. These plants were individually placed in gallon candy jars shown in plate 1, figure 2, in which they were grown for the remainder of the experimental period. Large plants with extensive root systems could be grown in these vessels. Both the 2-quart and the 4-quart culture vessels were covered with cardboard jackets to prevent the entrance of light.

A modified Tottingham solution,  $T_3R_1C_3$ , selected from those described by

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

Jones and Shive (3), was used for all cultures. The total osmotic concentrations of this solution was one atmosphere, and the partial volume-molecular concentrations of each of the four salts included were:  $\text{KH}_2\text{PO}_4$  — .00633 *M*;  $\text{Ca}(\text{NO}_3)_2$  — .00438 *M*;  $\text{MgSO}_4$  — .00711 *M*; and  $(\text{NH}_4)_2\text{SO}_4$  — .0014 *M*. This solution was one which had produced excellent growth of tomatoes in previous experiments. Supplements of boron in the form of boric acid and of manganese in the form of manganese sulfate were added to the culture solution in quantities sufficient to give a concentration of 0.5 p.p.m. of these elements. Freshly prepared ferrous sulfate in solution form was regularly added to the solutions at the rate of 0.5 p.p.m.

The solutions were renewed several times while the plants were still very small, but after 10 days, continuous renewal of the solutions was begun, the method used being that devised by Shive and Stahl (4). The rate of renewal while the plants were small was 1 liter every 24 hours.

At the same time that continuous renewal of the solutions was started, continuous aeration of one-half of the culture solutions was begun. The method of aeration was similar to that described by Allison (1), four or five bubbles of air per second escaping from the tube at the bottom of each culture jar. This aeration proceeded continuously until the time of harvest in all those cultures designated as "aerated." The cultures designated as "non-aerated" were not totally restricted to the oxygen normally dissolved in the solution, since the method of renewal employed (4) provides a small bubble of air for every drop of solution falling into the culture jar.

The plants in series A were placed in the culture vessels on July 13, 1930, three plants in each culture. They were harvested on August 25, and the green and dry weights of tops and roots were obtained in the usual way.

Series B and C were grown at a different season in 1931. The seedlings of series B were placed in the culture solutions on March 14 and were grown for 48 days, when they were harvested. The cultures of series C were also begun on March 14, but they were not harvested until they had been grown for 81 days. On account of the high rate of transpiration the rate of continuous solution renewal was increased on April 12 from 1 liter to 2 liters daily. On April 24 the osmotic concentration of the solutions of series C was reduced from 1 atmosphere to 0.5 atmosphere. Plate 1 shows photographs of plants 48 days old.

#### EXPERIMENTAL RESULTS

The data given in table 1 represent the average yields of plants grown in aerated and non-aerated solutions. These data clearly show the beneficial effect which aeration of the culture solution exerted upon the growth of both tops and roots of the plants. It will be observed that in each of the three series the average total dry weight yields of the cultures grown in aerated solutions were more than 50 per cent higher than the corresponding yields of those grown in the non-aerated solutions. The superior development of both tops and roots of the aerated plants is also clearly shown in plate 1.

This comparison between the cultures grown in the aerated and those grown in the non-aerated solutions is emphasized and quantitatively expressed by the data presented in table 2. These data represent the ratios of dry weight yields of the aerated to those of the non-aerated cultures grown under otherwise identical conditions. The markedly superior growth of both the tops and the roots of the aerated cultures is indicated by the high values of these ratios. The extremely high ratio of top growth given for the 48-day plants in series B (plate 1, fig. 1) is probably both an indication of the marked benefit from

TABLE 1

*Green and dry weights of tomato plants grown in aerated and non-aerated culture solutions*

CULTURES	AVERAGE WEIGHT PER PLANT			
	Green weight of tops	Dry weight of tops	Dry weight of roots	Total dry weight
	gm	gm.	gm.	gm.
Series A, 48 days:				
Non-aerated.....	22.4	1 8	0 2	2 0
Aerated.....	41 0	3 0	0 3	3 3
Series B, 48 days:				
Non-aerated.....	45 5	5 8	0 9	6 7
Aerated.....	157 7	16 0	1 7	17.7
Series C, 81 days:				
Non-aerated.....	280 1	34 0	4 8	38 8
Aerated.....	537 3	63 1	7.4	70 5

TABLE 2

*Ratios of dry weights of tops and roots and top/root ratios of tomato plants grown in aerated and non-aerated culture solutions*

CULTURES	DRY WEIGHT RATIOS—AERATED/ NON-AERATED CULTURES			DRY WEIGHT RATIOS— TOPS/ROOTS	
	Tops	Roots	Total	Non-aerated	Aerated
Series A, 48 days.....	1 67	1.50	1 65	9 0	10 0
Series B, 48 days.....	2 76	1 89	2 64	6 4	9 4
Series C, 81 days.....	1 86	1.54	1 82	7 1	8 5

aeration in crowded cultures and of the retarding effect upon growth of a deficient oxygen supply when containers are too small to accommodate adequately vigorous root systems. Three plants were included in each one of these cultures and the external environment at this season of the year was such as to promote excellent growth under these conditions. Lack of aeration of the culture solution, and in consequence an insufficient oxygen supply, is a very serious hindrance to normal growth and development and a potent factor in rendering worthless experimental data obtained from solution culture investigations.



The ratios of tops to roots given in table 2 emphasize a conclusion which is already suggested by the data in columns 1 and 2 of this table. It will be observed that these ratios of average dry weights in each of the three series are considerably higher for the aerated than they are for the corresponding non-aerated cultures, thus indicating that the influence of culture solution aeration upon top growth is relatively more pronounced than it is upon root growth. This relates, of course, to the tomato plant grown under the particular set of experimental conditions here specified.

Although the plants in the non-aerated solutions were much smaller, they started to blossom and to fruit earlier than did those in the aerated cultures. For this reason, the average total green weight of the fruits on plants of series C grown in the non-aerated solutions was somewhat greater at the time when harvested than that of the plants grown in the aerated solutions, the yield values being 399.7 and 321.0 gm. respectively. The weight of the fruit given does not represent the weight of fruit produced by a fully matured plant, of course, but simply shows the weight of the immature fruit already produced at this particular stage of development. However, at the time of harvest (81 days) the aerated plants showed every evidence of yielding a much larger crop of fruit than the non-aerated plants could yield.

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#### PLATE 1

INFLUENCE OF AERATION UPON ROOT AND TOP GROWTH OF TOMATOES IN SOLUTION CULTURES



FIG. 1. TYPICAL CULTURES FROM SERIES B

Left, aerated; right, non-aerated



FIG. 2. TYPICAL CULTURES FROM SERIES C

Left, aerated; right, non-aerated



# CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: I. ON THE SYNTHESIS OF THE "HUMUS NUCLEUS"<sup>1</sup>

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The subject of the chemistry of humus, or the dark colored organic matter in soils, peats, and composts, has attracted considerable attention during the last 150 years. Although chemists and agronomists have frequently used the terms "humus," "humic acid," "ulmic acid," "humin," "ulmin," and numerous others to designate complexes with seemingly different chemical properties, it has become now generally recognized that all these preparations were not definite compounds but depended largely upon the procedures used for their extraction. All these dark colored organic complexes are left in the soil or are formed there as a result of the decomposition of plant and animal residues. The nature of the soil, the nature of the residues, and the conditions of decomposition largely influence their chemical nature.

Numerous attempts have also been made since 1830 to synthesize by chemical procedures dark colored organic complexes which would have the same properties as the humus of the soil, of peat, and of decomposing plant residues (23). The dark color of the soil humus, its solubility in dilute alkalis, and its precipitation by acids, as well as its relatively high carbon content, served as a basis for these attempted syntheses; numerous complexes were thus prepared having properties similar to those of the humus in soils and composts. The treatments consisted in the action of acids at high temperatures on carbohydrates, the interaction of sugars with amino acids, and the oxidation of phenol and its derivatives. The dark colored preparations formed under these conditions cannot be compared with full justification with those formed under natural conditions. The natural products are markedly different, both because of the difference in the initial substances from which the humus is formed and because of the nature of the processes whereby this is brought about. There are also marked chemical differences which are not always taken into consideration, such as the relatively constant nitrogen content of the natural preparations. All the attempts to synthesize humus by chemical agencies may be considered to have failed so far in giving preparations which would be identical in all respects with the humus formed under natural conditions; the information thus accumulated may be considered to have had no influence

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upon our knowledge of the chemical nature of humus in soils and composts. Actually the syntheses tended to confuse the subject rather than to clarify it and explain the processes governing the formation of humus, its nature, and its decomposition.

This failure to synthesize complexes comparable to soil humus is due largely to the fact that, in all the attempts to obtain dark colored substances by treatment of organic complexes with chemical reagents, no consideration was given to the natural processes which are responsible for the formation of humus from plant residues, namely, the action of microorganisms. Although these investigations were begun at a time when microbiology, especially the microbiology of the soil, was non-existent, they have been considered in the same manner during the last 50 years, which witnessed an extensive development of our knowledge of the rôle of microorganisms in soil processes. As a result of this, a century of progress of organic chemistry has contributed little to the knowledge of the chemical composition of the dark colored organic complexes of the soil. To be sure, considerable information has accumulated concerning the physico-chemical properties of humus (17); it has also been shown that humus is not so simple in chemical composition as originally assumed and that numerous substances can be isolated from it (19). These were the only positive phases of humus investigation, outside, of course, of the accumulated knowledge concerning the rôle of humus in soil processes and in the growth of plants and microorganisms. However, the fundamental problem still remained unsolved, namely, the origin and chemical nature of the basic complex which makes up the soil humus. One would hardly be justified in considering the continuously increasing number of "humic acids," which were in most instances labels for different preparations rather than for definite chemical entities, as contributing to the advance of our knowledge of the soil humus. Here the ideas have not progressed much from those in vogue a century ago, with a mere substitution of new names for old ones.

With the advance of our knowledge of the chemical composition of plant and animal residues, with the growing appreciation of the biochemical processes carried out by soil microorganisms and with the recognition of the importance of these microorganisms in the decomposition of plant and animal residues, especially when it was found that not all the chemical constituents of those residues decompose at a uniform rate, the chemical nature of humus and the processes leading to its formation have become better understood. Of special importance in this connection has been the recognition of the fact that, among the plant constituents, the lignins are more resistant than the other organic complexes to decomposition by fungi and bacteria, which are responsible for the transformation of plant residues in soil, peat, and composts. The accumulated information regarding the large quantities of proteins and other organic nitrogenous complexes which are synthesized during the decomposition of the plant residues, has further contributed to our knowledge of the nature and formation of humus.

These two complexes, namely, the lignins and the proteins, make up as much as 70 to 80 per cent of the humus in mineral soils (25, 29); in lowmoor, forest, and sedimentary peats, as well as in composts which have undergone considerable decomposition. The increase in these two chemical constituents of the humus accounts for its high carbon and nitrogen content. This is due primarily to the high carbon content of the lignin and to the high nitrogen content of the protein. Further, the fact that a definite carbon-nitrogen ratio, approaching 10 to 1, has been found for the organic matter or humus of many mineral soils points to the possibility of the existence of a certain chemical relationship between the lignin and the protein groups. The fact, however, that this carbon-nitrogen ratio is frequently modified, as by increasing depth of soil, by varying climatic conditions, and by the nature of the plant residues and their decomposition, tends to show that if such chemical relations exist they are not absolute but are variously modified.

One must also emphasize the fact that, in addition to the lignin-like complexes (or lignin derivatives) and the protein-like complexes, humus contains also a number of other organic substances, most important of which are certain hemicelluloses, largely uronides, certain fats and waxes, higher alcohols, and organic acids. The nature and abundance of these compounds depend upon the nature of the plant substances which contribute to the formation of the humus, the extent of their decomposition, the soil reaction, and climate.

Various attempts have recently been made to separate the organic matter of the soil (20) into "humus" and "non-humus" fractions, or into "humic" and "non-humic" bodies (22, 9), by the use of different reagents, such as acetyl bromide (12, 21) or hydrogen peroxide (15). Such separations, however, are not based upon a better knowledge of the chemical nature and origin of the soil organic matter than that done by the earlier investigators, who have separated this organic matter into "humins" and "humic acids," on the basis of their solubility in alkalis (2, 17). These procedures further emphasized the fact that the soil organic matter, to which alone the term "humus" can be justly applied, can be separated into alkali-soluble and alkali-insoluble complexes; the latter can be, however, further dissolved, partly at least, in hot alkali solutions. The alkali-soluble part gives, on neutralization with acids, a precipitate, while certain substances are left in solution. It is this alkali-soluble portion, after it has been precipitated by acids, which has attracted the greatest attention, since it forms a large part (30 to 60 per cent) of the humus in soils and in peats. It is also this fraction which seems to be more resistant to further decomposition than the other humus constituents. It is this fraction which resembles in its properties lignin, and to which the terms "humus" and "humified portion" have frequently been attached. Several theories have been recently suggested (5, 23) in which lignin was considered to be the mother substance of "humus" or the alkali-soluble and acid-precipitated part of humus.

The fact that lignin and other plant constituents are largely soluble in acetyl-bromide, while a part of the humus is insoluble, merely proves that in

the decomposition of the plant residues, certain substances are formed, either by the transformation of some of the original plant constituents or through processes of synthesis by microorganisms, which are not acted upon by this reagent.

Although it is usually stated that humus is highly resistant to decomposition, actually it decomposes very slowly, otherwise its accumulation in the soil would increase indefinitely; under conditions of continuous cultivation, the humus content of the soil is gradually diminished. Various microorganisms, especially certain fungi, are known to be capable of decomposing lignins; there is no reason, therefore, to assume that these organisms should not be able to decompose as well the complexes which may be formed from lignin, either as a result of its transformation or combination with other plant, animal, or microbial constituents. In view of the resistance of the proteins in the humus to rapid decomposition, it was suggested that possibly a lignin-protein complex is formed which renders the proteins less readily subjected to attack by microorganisms.

Dehérain (3) observed the fact that the humus of manure is only moderately acted upon by microorganisms, liberating the nitrogen very slowly, in the form of nitrate; he suggested that this humus may be a mixture of lignin and protein, the latter being synthesized by bacteria. In view of the fact, however, that ordinary plant and animal proteins are readily attacked by bacteria, one could not be dealing here with an ordinary mechanical mixture of lignins and proteins, but with chemical compounds. Another suggestion was that the organic nitrogenous complexes in the humus are of a different chemical nature than common plant and animal proteins (1, 11). Lathrop (13) found previously that whatever the chemical nature of the proteins added to the soil, as shown by the distribution of amino groups, their decomposition leads to the formation of a new protein complex.

According to Hobson (10), the "humic acid" complexes of the soil may be regarded as adsorption compounds between lignins and proteins; the latter are thereby protected against bacterial attack. Alkali solutions of lignin and albumin were mixed and precipitated together by an acid; the amino nitrogen distribution (by the Van Slyke procedure) in this complex was found to be identical to that of the "humic acid" isolated from the soil. Jensen (11) also mixed alkali solutions of lignin and casein and precipitated the mixture with hydrochloric acid; the preparations, containing 3.9–4.1 per cent of nitrogen, were added to nutrient solutions containing the necessary minerals. These were inoculated with soil suspensions and incubated at 25°C. for 3 weeks; only traces of ammonia were formed in these solutions, but the control solution, containing a corresponding amount of nitrogen as casein, gave a transformation of 75–80 per cent of the nitrogen to ammonia. However, in the soil the resistance of this compound of lignin and casein to microbial attack was not so marked, and after 45 days only an insignificant part of the nitrogen was recovered as humus. Jensen concluded that lignin does not seem to be able to form such resistant adsorption compounds with every protein.

Demolon and Brigando (4) found that arable soils are able to fix dissolved proteins brought into contact with these soils; the protein thus fixed by the humus colloids could be nitrified in the soil just as readily as the pure proteins. The humic nitrogen, however, was not nitrified, thus leading to the conclusion that the stability of humic nitrogen in the soil is due to its specific chemical composition.

The rôle of tannins in rendering the organic nitrogenous compounds in humus more resistant to decomposition has also been suggested. Wollny (33) found that the decomposition of protein-rich soybean leaves is markedly delayed, as measured by the  $\text{CO}_2$  evolution, when the material is moistened with a tannin solution; this was explained as due to the formation of a resistant tannin-protein complex. Moeller (16) considered the interaction of tannins with proteins as leading to the formation of "humus bodies." The gradual disappearance of the tannins in the process of decomposition of wood was explained by the formation of "humic acids," whereby the tannin thus combined no longer gives the tannin reaction and cannot be extracted with water. Tannin was found (14) to precipitate completely egg albumen, casein, milk protein, and other proteins; the ratio between the tannin and the nitrogen in the form of protein was about 20:1. Weis (30) has shown previously that albumoses and peptone are also completely precipitated by tannin. The problem remained to determine whether lignin and protein also form similar complexes.

The average chemical composition of the organic matter of six soils (25, 29) gave 43.27 per cent lignin ("lignin-humus complex" or "soil lignin"), 33.81 per cent protein or organic nitrogenous complexes, 11.02 per cent water-insoluble carbohydrates (cellulose, hemicelluloses, etc.), and 2.77 per cent ether and alcohol-soluble substances, thus accounting for 90.87 per cent of the total organic matter of the soil. Of this organic matter accounted for, 78.08 per cent was made up of two complexes, namely, the protein and the lignin. The same is true of lowmoor and sedimentary peats (26) and of well-decomposed manure (27). We are justified in concluding that the humus of mineral soils, of well-decomposed peats, and of composts consists predominantly of two chemical complexes, namely, lignins and proteins, with an admixture of other substances, such as fats and waxes, cellulose and hemicelluloses, and organic acids and alcohols; the nature and abundance of these accompanying substances depend upon the nature of the plant and animal residues added to the soil or used in making the compost, upon the extent of their decomposition, and upon the conditions under which this decomposition is taking place, such as reaction, moisture content, aeration, and temperature. Whether the two major complexes, namely, the lignins and the proteins, form one chemical complex in the humus, or whether they exist independently still remains to be determined.

The chemistry of humus thus resolves itself into a knowledge of the chemistry of two groups of substances, one group comprising the lignin and protein complexes, on the one hand, and another group comprising the remaining



organic complexes, on the other. This may be looked upon as a more modern paraphrase of the earlier conception of "humic acids," on the one hand, and of "humin" and "crenic" or "fulvic acids," on the other; or of the more recent conception of "humic substances," on the one hand, and of "non-humic compounds," on the other. This idea thus has a true historical background.

The subject of this contribution is to show that the humus of the soil consists of a major complex, which may be called, for want of a better name, "humus-nucleus" (28), consisting of the lignin and protein fractions, having certain chemical affinities; this complex is accompanied in the soil humus by other substances, either left from the decomposition of the plant and animal residues, or synthesized through the activities of the microorganisms. This complex was found to possess all the biological, chemical, and physical properties of the major fraction of the humus of the soil and is largely equivalent to the "humic acids" or "humic bodies." The term "humus nucleus" is not the happiest one, since the authors do not mean thereby a nucleus, in the grammatical sense, but merely the major fraction of humus in soils, peats, and composts. The term "synthetic humus" would have been more applicable, but since the latter has been so often applied, since the time of Malaguti, to dark colored substances formed by the treatment of carbohydrates with acids and with other chemical reagents, processes which have no equivalent under natural soil conditions, it is purposely avoided.

In carrying out these experiments, an attempt was always made to avoid as much as possible the use of high temperatures or of excessive treatment of the materials with chemical reagents. The idea was to establish: 1. what effect the presence of lignin has upon the decomposition of proteins and protein derivatives by microorganisms; 2. whether a stable (chemical ?) complex is formed between lignin and protein; 3. how this complex compares in the rapidity and nature of decomposition with similar complexes found in soils and in peats; 4. the comparative chemical and physico-chemical behavior of the two constituents; 5. what influence the synthesized complex has upon various important soil processes carried out by microorganisms.

#### EXPERIMENTAL

##### *Preparation of lignin and proteins*

Lignin has been defined by Wilstätter (31) as the unsaccharified part of the plant membrane. According to Wislicenus (32), lignin is "the sum of highly molecular, colloiddally soluble bodies which are absorbed on the surface of cellulose fibers from the plant sap or cambium sap." These two definitions, to which numerous others could be added, are sufficient to illustrate the vagueness still existing concerning the nature of this important plant constituent. Chemically, lignin is considered by most chemists as a polymer of a complex which consists of a benzol ring, with several hydroxyl (OH), methoxyl (OCH<sub>3</sub>), and carboxyl (COOH) groups. It was at first assumed that the methoxyl content of lignin is constant and lignin was frequently measured

quantitatively in plant residues and in soil by the amount of methoxyl present; more recent investigations, however, have shown that not only does the methoxyl content of lignin vary in different plant materials and even in different parts of the same material (18), but that in the decomposition of lignin by microorganisms, the methoxyl may be destroyed more rapidly than the rest of the molecule. A detailed study of the chemistry of lignin is found in the work of Fuchs (7), Freudenberg (6), and others. Attention should be called here to one characteristic property of lignin, namely, its solubility in alkali solutions, and the formation of definite chemical or adsorption complexes with bases. Proteins are amphoteric electrolytes and can combine with both acids and bases.

For the preparation of lignin from plant materials, a number of methods are at present available. There is no doubt that lignin obtained by different procedures varies in its chemical composition. The two methods most commonly employed are the following:

1. The treatment of the plant material in the cold with concentrated acids, such as 42 per cent hydrochloric or 66 to 80 per cent sulfuric; by this treatment the cellulose and other carbohydrates are brought into solution, and the lignin is left free as "acid lignin;" if the plant material is low in protein and in ash, as in the case of wood or straw, and the fats and waxes are removed by preliminary treatment with ether, a fairly uniform product is obtained. 2. The extraction of the plant material with sodium or potassium hydroxide solution, at a high temperature, thereby bringing the lignin into solution; on acidifying the alkali solution, the lignin is precipitated; if the precipitate is now boiled with an excess of the dilute acid, those hemicelluloses which were removed with the lignin will be hydrolyzed and brought into solution, the precipitate is filtered off and washed, giving fairly pure lignin, which is referred to as "alkali-lignin."

The yields obtained by the two procedures differ considerably, the amount of acid lignin obtained from the same plant material being considerably greater than that of alkali lignin.

For the following experiments, alkali lignin from wheat straw was prepared by treating the straw first with hot water and with hot dilute hydrochloric acid to remove the water-soluble substances, the starches, and a large part of the hemicelluloses and proteins. The residual material was then extracted with aqueous 4 per cent NaOH solution at 120°C. for 5 hours; the extract was filtered off and the residue again extracted with fresh alkali solution. The combined extracts were neutralized with HCl, made slightly acid, and boiled to remove the extracted hemicelluloses and as much of the nitrogenous complexes as came down with the lignin. The lignin was now washed with water until free from acid and dried at 70°C. The air-dried lignin preparation had the following composition:

	<i>per cent</i>
Moisture.....	3 00
Ash.....	0 08
Nitrogen.....	0.25
Hemicellulose.....	0 85
Free lignin.....	94 45

When treated with 10 volumes of 80 per cent sulfuric acid for several hours in the cold, then diluted with 15 volumes of water and steamed for 5 hours, 90 per cent of this alkali lignin was found to be equivalent to acid lignin. In some of the experiments on the influence of lignin upon protein decomposition by microorganisms, acid lignin, prepared from straw or from wood, was also used.

As a source of protein, casein purified according to the method of Hammarsten, gliadin prepared from wheat flour by the well-known procedures, and egg-albumen were employed. The casein contained 9.3 per cent moisture and 15.87 per cent nitrogen on a dry basis, and the gliadin contained 5.5 per cent moisture and 15.35 per cent nitrogen on a dry basis.

*Influence of lignin upon the decomposition of proteins by microorganisms*

Four parts of lignin and one part of protein were thoroughly mixed in a dry state and aliquot portions (to give 1 per cent of protein) of the mixture added

TABLE 1

*Influence of lignin upon the decomposition of proteins by pure and mixed cultures of microorganisms*

Nitrogen liberated as ammonia in 11 days from 500 mgm. of protein material.

ORGANISM	NITROGEN LIBERATED AS AMMONIA FROM			
	Casein alone	Casein + lignin	Gliadin alone	Gliadin + lignin
	mgm.	mgm.	mgm.	mgm.
Control. ....	0 28	1 26	0 56	1.12
Soil infusion.....	40.88	20.30	37 21	25.48
<i>Bac. cereus</i> .....	36 82	4 20	31.50	3 64
<i>Actinomyces</i> sp.....	18 87	1 40	18 34	1 12
<i>Trichoderma</i> sp.....	21 56	22 26	18 06	16 47

to 50-cc. portions of a liquid medium; the latter was sterilized, inoculated either with a soil infusion or with pure cultures of microorganisms, and incubated for 11 days, at the end of which time the ammonia-nitrogen was determined by distillation with MgO. The basic medium consisted of:

$K_2HPO_4$ .....	1.0 gm.
$MgSO_4 \cdot 7H_2O$ .....	0.5 gm.
NaCl.....	0.1 gm.
$FeSO_4$ .....	0.2 gm.
Distilled water.....	1,000 cc.
Reaction.....	pH 7.0

The results presented in table 1 show that a mere mechanical admixture of lignin and protein had, in many cases, a marked depressing effect upon ammonia formation. Even the mixed soil microbial population liberated only about a half as much nitrogen, from the decomposition of the proteins, in the presence of lignin as in its absence. In the case of the pure cultures of the

bacterium and actinomyces, the depressive effect of the lignin was especially marked, since the protein decomposition, as measured by ammonia liberation, was reduced to a minimum and frequently almost depressed entirely. However, in the case of the fungus, the presence of lignin exerted no injurious effect at all, or was very limited.

In order to determine whether it is the property of only the alkali lignin to depress the decomposition of the protein, or of lignin in general, acid lignin and alkali lignin were used in the following experiment. Varying amounts of two proteins and of the two lignins were added to 100-gm. portions of washed

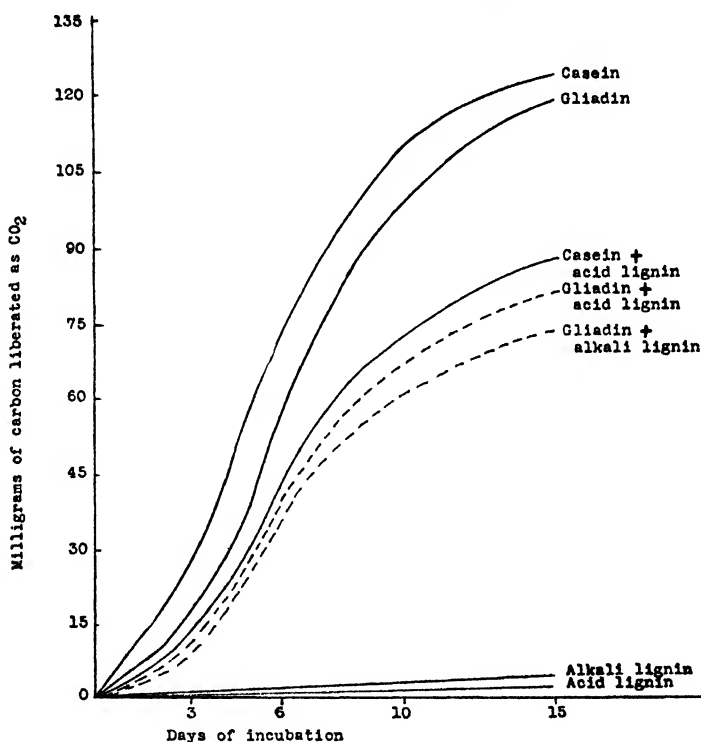


FIG. 1. INFLUENCE OF ACID-LIGNIN AND ALKALI-LIGNIN UPON THE DECOMPOSITION OF CASEIN AND GLIADIN BY MICROORGANISMS IN SAND MEDIUM, AS SHOWN BY THE LIBERATION OF CO<sub>2</sub>

sand containing sufficient moisture to make conditions aerobic; various inorganic salts were also added. When protein and lignin were employed, the two were mixed mechanically by hand. The cultures were inoculated with soil infusion and incubated for 15 days. The flasks were connected with a respirator so as to measure the course of evolution of CO<sub>2</sub> during the decomposition of the protein, in the presence and absence of lignin. At the end of the incubation period, the ammonia produced was determined in an aliquot portion of the culture. To establish whether the protein has combined with the lignin

to form a "humus" complex ( $\alpha$ -humus) (24), the residual material was extracted with 4 per cent NaOH solution; the extract was precipitated with HCl, filtered, washed, dried, and weighed; the nitrogen content of this "humus" was then determined. The results obtained are presented in table 2 and fig. 1. These results bring out very definitely the fact that both acid and alkali lignin have a marked depressive effect upon the decomposition of proteins in sand media, under favorable conditions and by a mixed microbial soil population. Further, the results point definitely to the formation of a complex between lignin and protein; this complex is equivalent to "humic acid" or to  $\alpha$ -humus, in its solubility in alkalis and precipitation by acids, as well as in its nitrogen content. The amount of nitrogen found in this complex is nearly equivalent to the reduction in the amount of protein decomposed, in the presence of the lignin, as shown by the lower ammonia formation.

TABLE 2

*Influence of acid and alkali lignin upon the decomposition of proteins in sand medium\**

SOURCE OF PROTEIN†	SOURCE OF LIGNIN	CONCENTRATION OF LIGNIN	CARBON DIOXIDE EVOLUTION, AS CARBON	NITROGEN LIBERATION, AS AMMONIA	$\alpha$ -HUMUS IN RESIDUAL MATERIAL	NITROGEN CONTENT OF $\alpha$ -HUMUS
		gm.	mgm.	mgm.	gm.	mgm.
Casein	0	...	121.7	56.70	0.048	1.82
Casein	Acid lignin	2.5	85.0	44.80	0.415	10.24
Gliadin	0	..	120.5	54.88	0.055	1.68
Gliadin	Alkali lignin	2.5	72.9	42.42	1.033	18.09
Gliadin	Acid lignin	2.5	81.3	44.80	0.395	10.54
0	Alkali lignin	1.0	3.1	0.56	0.400	1.12
0	Acid lignin	1.0	2.7	0.28	0.185	0.98

\* Incubation 15 days; mixed soil infusion used for inoculation of all cultures.

† One-half gram of protein used.

In the following experiment, the casein and lignin were dissolved in 4 per cent NaOH solution (by warming), the solutions were mixed, so as to give 3 parts of lignin to 1 part of casein, and the mixture was acidified with hydrochloric acid. The precipitate formed was filtered off, washed with water until free from acid, and dried. The complex, in quantities equivalent to 500 mgm. of casein, was added to solution media similar to those described in the foregoing and to sand media, containing optimum moisture so as to give aerobic conditions and the necessary inorganic nutrients; the media were sterilized, inoculated, and incubated for 11 days. The results given in table 3 show that both in liquid and in sand media there was ~~no~~ practically no decomposition of the casein, present in the form of the lignin-casein complex, as shown by ammonia liberation. This experiment was repeated, using other preparations and a 30-day period of incubation, with similar results.

To throw further light upon the effect of varying amounts of lignin and

upon the influence of sterilization of the medium upon the decomposition of the protein, the results obtained in the following experiment are reported. One-half gram portions of casein and varying amounts of lignin were added to 50-cc. quantities of solution medium containing the mineral salts; in some cases, the casein was used in the form of the lignin-casein complex, prepared

TABLE 3

*Decomposition of lignin-casein complex by pure and mixed cultures of microorganisms*

Nitrogen liberated as ammonia from 2 gm. of the complex containing about 500 mgm. of

ORGANISM	NITROGEN LIBERATED AS AMMONIA FROM	
	Liquid medium	Sand medium
	mgm.	mgm.
Control . . . . .	1 12	0 70
Soil infusion. . . . .	2 52	1 96
<i>Bac. cereus</i> . . . . .	1 12	0 84
<i>Actinomyces</i> sp. . . . .	1 12	0 70
<i>Trichoderma</i> sp. . . . .	1 12	0 84

TABLE 4

*Influence of varying quantities of lignin upon the decomposition of casein by a mixture of soil microorganisms*

Ammonia liberated from 500 mgm. of casein

AMOUNT OF LIGNIN USED	TREATMENT	STERILIZED	AMMONIA-NITROGEN FORMED
gm.			mgm.
None	Casein	+	42 0
None	Casein alone	—	42.4
1	Casein and lignin mixed mechanically	+	2.52
1	Casein and lignin mixed mechanically	—	31 72
2	Casein and lignin mixed mechanically	+	2 24
2	Casein and lignin mixed mechanically	—	23.75
2	Casein and lignin dissolved in alkali solutions, mixed in the cold and precipitated with acid	+	1.68
2	Casein and lignin dissolved in alkali solutions, mixed in the cold and precipitated with acid	—	1.68

according to the procedure outlined in the foregoing, four parts of lignin being used to one part of casein. Some of the cultures were sterilized and others were left unsterilized. All the cultures were inoculated with soil infusion. At the end of 11 days' incubation, the ammonia nitrogen was determined (table 4). The results show that the mere addition of lignin to the casein had a depressing effect upon the decomposition of the latter, as measured by

ammonia liberation, this effect increasing with an increase in the relative concentration of the lignin. When the medium was sterilized, the depressing effect was especially marked, since the heating resulted in the caking up of the lignin and led to the formation of a casein-lignin complex. When such a complex was produced by mixing alkali solutions of the two materials in the cold, and reprecipitating the complex by means of acid, the decomposition of the casein was stopped almost completely.

In order to obtain further information on the influence of increasing quantities of lignin upon the decomposition of casein, varying quantities of the two complexes were mixed in the form of dry powders and introduced into 50-cc. portions of the liquid medium. The media were left unsterilized, and were inoculated with a soil infusion. After 11 days' incubation at 28°C., the amounts of ammonia produced were determined (table 5). The results prove quite conclusively that increasing concentrations of lignin have a depressing effect upon the decomposition of the casein and upon the liberation of the

TABLE 5  
*Influence of increasing amounts of lignin upon the decomposition of casein by a mixed soil population*

CASEIN USED	LIGNIN USED	AMMONIA-NITROGEN LIBERATED
gm.	gm.	mgm.
0.5	0	44.52
0.5	0.5	39.20
0.5	1.0	35.70
0.5	2.0	29.82
0.5	4.0	15.96
0.5	8.0	12.74

nitrogen in the form of ammonia by a mixed soil population, even when the medium is left unsterilized.

The following experiment deals also with the influence of varying concentrations of lignin upon the decomposition of casein by a mixed soil population in a non-sterilized liquid medium (50 cc.), containing inorganic salts and adjusted to pH 7.0. The two complexes were dissolved in normal solutions of sodium hydroxide and mixed in varying proportions. The mixed solutions were acidified to pH 4.5, which resulted in the formation of a heavy precipitate. This precipitate was filtered off, washed, dried at a low temperature, and used for the decomposition studies. In all cases sufficient casein solution was used to give 500 mgm. of the air-dry casein; however, when the alkali solution of the casein was adjusted to pH 4.5 by hydrochloric acid, not all the casein was precipitated out; the greater the relative amount of lignin used, the greater was the quantity of casein brought down in the complex, as shown by the total nitrogen content of the latter. The decomposition was allowed to proceed for 11 days and the ammonia was then determined.

The results (table 6) show definitely that the formation of a ligno-casein complex has a marked depressive effect upon the decomposition of the casein and the liberation of the nitrogen as ammonia. The greater the relative concentration of the lignin, when the protein concentration is kept constant, the less is the relative decomposition of the protein. The formation of a complex between the casein and the lignin is brought out by the fact that with an increasing relative concentration of lignin, the amount of casein removed from solution was increased. It remains to be determined whether we are dealing here with a physical or adsorption complex or with a chemical complex possessing new properties, the most important of which is the increasing resistance of the lignin to decomposition by microorganisms. Even when the lignin concentration was only one-fifteenth or one-tenth of the amount of casein in solu-

TABLE 6

*Influence of varying concentrations of lignin and casein in the casein-lignin complex upon its decomposition by a mixed soil microbial population*

COMPOSITION OF COMPLEX		YIELD OF PRECIPITATE	TOTAL NITROGEN IN COMPLEX*	AMMONIA-N LIBERATED	PER CENT OF TOTAL NITROGEN IN THE CASEIN LIBERATED AS AMMONIA
Casein used	Lignin used				
gm.	gm	gm.	mgm.	mgm.	
0 500	0	0 200	21 56	8 40	39 0
0 500	0 010	0 232	23 80	8 12	34.1
0 500	0 050	0 247	23 88	7 70	32 2
0 500	0 200	0 517	32 48	6 58	20 3
0 500	0 500	0.849	41 25	2 80	6 8
0 500	1 250	1.540	43.50	0 14	0
0 500	2 500	2.750	47 60	0 14	0
0 500	5 000	5 224	56 45	0 14	0
0	0 500	0 501	2 80	0 14	0

\* The nitrogen derived from the casein; the small amount of nitrogen present in the lignin was subtracted.

tion, there was already a sufficiently marked effect to reduce the casein decomposition, as measured by the liberation of nitrogen as ammonia. When the concentration of lignin was equivalent to that of the casein, the rate of decomposition of the latter was reduced to one-sixth of the relative rate of decomposition of the casein alone. When the concentration of lignin was two and a half times as great as that of casein, the decomposition of the latter was stopped completely.

There is no doubt that the protein can be absorbed from its solution by lignin, as shown in table 7. In this experiment, 50-cc. portions of casein solutions, equivalent to 1 gm. of air-dry casein, were adjusted to near neutrality and treated with varying quantities of moist lignin, which had been washed practically free from acid (pH 4.0) but not dried. The suspension of lignin and casein was shaken and allowed to stand in the cold for several hours,



filtered, washed, and the amount of nitrogen removed from the solution in the form of protein determined. The results (table 7) show that this is nearly proportional to the amount of lignin used.

It was essential to determine whether lignin has a depressive effect upon the decomposition of protein degradation products, such as peptones, polypeptides, and amino acids, in a manner similar to that exerted upon the decomposition of native proteins. This problem, apart from its theoretical significance, has also important practical applications, as in the decomposition of stable manure and green manures, in which a large part of the nitrogen is

TABLE 7

*Absorption of protein from neutral solution by different amounts of alkali lignin in the cold*

Fifty cubic centimeters of casein solution containing 1 gm. of air-dry casein was used in all cases.

AMOUNT OF LIGNIN USED <i>gm.*</i>	REACTION OF CASEIN SOLUTION OR OF FILTRATE <i>pH</i>	CASEIN REMOVED	
		<i>mgm N</i>	<i>per cent of total N</i>
0	7 15	0	0
1	6 20	22 9	15 8
2	6 06	37 1	25 6
3	5 96	48 3	33 3

\* On dry basis.

TABLE 8

*Influence of lignin upon the decomposition of peptone and amino acids by microorganisms*

NITROGEN SOURCE	LIGNIN <i>gm.</i>	CARBON LIBERATED AS CO <sub>2</sub> <i>mgm</i>	NITROGEN LIBERATED AS AMMONIA <i>mgm</i>
1 gm. peptone .....	0	238 6	98 35
1 gm. peptone.....	4	243 6	94 85
1 gm. glutamic acid.....	0	241 9	71.05
1 gm. glutamic acid .....	4	243.0	69.65
1 gm. glycocoll .....	0	75 3	83 85
1 gm. glycocoll.....	4	156 7	123 55

present in the form of simple protein degradation products or protein building stones. Definite amounts of alkali lignin were mixed with peptone (Difco), glutamic acid, or glycocoll, in the proportion of four parts of lignin to one part of the nitrogenous complexes. The mixtures were placed in flasks containing 100-gm. portions of sand and the necessary inorganic salts, inoculated with a soil infusion, and connected with the respiration apparatus. The amount of CO<sub>2</sub> liberated in the process of decomposition was measured. At the end of the incubation period (21 days), the ammonia formed was determined (table 8).

The resultant effect of lignin upon the decomposition of peptone, as measured by evolution of CO<sub>2</sub> and accumulation of ammonia was neutral, i.e.

neither injurious nor beneficial. The same was true of its effect upon the decomposition of glutamic acid; however, in the case of glycocoll decomposition, the effect of lignin was beneficial. In this respect, the action of the lignin upon the decomposition of amino acids was similar to that exerted upon other microbiological processes, as reported later. These results thus prove beyond any doubt that the injurious effect of lignin upon protein decomposition holds true only for native proteins.

*Synthesis of ligno-protein complexes and their decomposition*

The results already presented seem to point to the formation between lignin and protein of complexes, which have properties distinctly different from both constituents. The purpose of the following experiments is to study the nature of these complexes, their formation under conditions similar to those which prevail in nature, their chemical and physico-chemical properties, their decom-

TABLE 9  
*Influence of reaction and bases upon the precipitation of the ligno-protein complex*

REACTION	BASE USED	WEIGHT OF PRECIPITATE*	TOTAL NITROGEN IN PRECIPITATE†
pH		gm.	mgm.
5.5	H <sup>+</sup>	2 620	56 10
5.0	H <sup>+</sup>	2 857	65 58
4.5	H <sup>+</sup>	2 895	72 50
4.0	H <sup>+</sup>	2 940	73 67
6.0	Mg <sup>++</sup>	3 615	60 70
6.0	Ca <sup>++</sup>	3 520	62 72
8.0	Ca <sup>++</sup>	3 580	62 15

\* Five hundred milligrams casein and 2.5 gm. of lignin used, air-dry material.

† The ash content was 1.4 per cent for the hydrogen preparation and 15.0 per cent for the calcium preparation; an iron complex was also prepared containing 4.6 per cent iron.

position, and their influence upon the growth and activities of microorganisms. The relation of these complexes to the various bases which commonly occur in soil presented a most interesting problem. In view of the fact that the reaction of the soil is definitely dependent upon the nature and concentration of the soil bases, the influence of reaction upon the precipitation of the ligno-protein complex, as well as the influence of ions of heavy metals, especially of Ca and Mg, upon the formation of this complex was determined. Alkali solutions of casein and lignin, in the ratio of 5 parts of lignin to 1 part of casein were mixed. Definite quantities of the two solutions were placed in a series of flasks and enough HCl added to adjust the reaction to pH 5.5, 5.0, 4.5, and 4.0, respectively. In the absence of salts, practically no precipitate was formed at pH 5.5 or above; at pH 4.0, practically the whole complex was precipitated out. When aqueous solutions of CaCl<sub>2</sub> and MgCl<sub>2</sub> were added, the whole ligno-casein complex was precipitated out at much lower hydrogen-

ion concentrations (pH 5.5–8.0). The precipitates were washed free from salts with water, dried, weighed, and analyzed for total nitrogen (table 9).

These preparations were added to liquid media containing mineral salts, inoculated with a soil suspension, and incubated. None of the cultures showed any signs of decomposition in 11 days, as measured by ammonia formation; very little microbial growth could be found by macroscopic and microscopic observation. This definitely pointed to the fact that, as a result of the interaction of lignin with protein, complexes had been formed consisting of a certain concentration of base, of lignin, and of protein; the protein was thus inactivated almost quantitatively against decomposition by microorganisms.

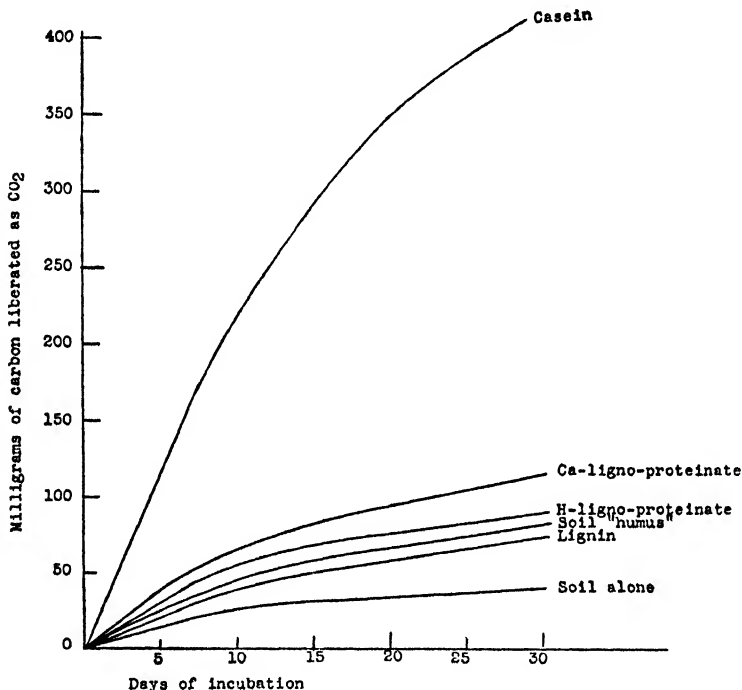


FIG. 2. COURSE OF DECOMPOSITION OF CASEIN, LIGNIN, SOIL HUMUS, AND LIGNO-PROTEIN COMPLEXES IN SOIL, AS MEASURED BY THE EVOLUTION OF CO<sub>2</sub>

The experiments on the decomposition of the ligno-protein complexes have been carried out so far in solution and in sand media, for comparatively short periods of time. It is essential of course to determine the behavior of these complexes in the soil. Two-hundred gram quantities of a Sassafra loam soil were placed in a series of Erlenmeyer flasks. Some of the soils were left untreated and only adjusted to optimum moisture content; some received 6-gm. portions of alkali lignin, some received 6-gm. portions of the H-ligno-protein complex, and some received 6-gm. portions of the Ca-ligno-protein complex. For the purpose of comparison, 4.5-gm. portions of  $\alpha$ -humus, ob-

tained from soil by extraction with alkali and precipitation with acid, were added to a series of flasks; some of the flasks received 0.95-gm. portions of pure casein. The nitrogen content of the casein used was 136.8 mgm., of the H-ligno-proteininate 136.08 mgm., of the Ca-ligno-proteininate 134.4 mgm., of the  $\alpha$ -humus 134.8 mgm., and of the lignin 20.16 mgm. Sufficient water was added to bring the soils to optimum moisture. The flasks were connected with the respiration apparatus and the amount of CO<sub>2</sub> evolved was determined (fig. 2). At the end of 30 days' incubation, the ammonia and nitrate contents in the soils were measured. A portion of the soil was extracted with 4 per cent sodium hydroxide solution, the extract filtered and the filtrate acidified with hydrochloric acid; the precipitate thus formed was removed, washed with water, and dried.

The results (table 10) show that free lignin was decomposed in the soil only to a limited extent; this decomposition was accompanied by a reduction in the

TABLE 10

*Decomposition of lignin and ligno-protein complexes in soil, as measured by CO<sub>2</sub> evolution, nitrogen liberation, and humus formation*

SOIL TREATMENT	CO <sub>2</sub> LIBERATION		NITROGEN LIBERATION			$\alpha$ -HUMUS	
	15 days	30 days	NH <sub>3</sub>	NO <sub>3</sub>	Total N	Yield	Excess over control
	mgm. C	mgm. C	mgm. N	mgm. N	mgm.	gm.	gm.
Soil alone. . . . .	29 8	41 0	0	8 96	8 96	1 304	. . .
Soil + lignin . . . . .	49 8	72 7	0	5 60	5 60	7 077	5 773
Soil + $\alpha$ -humus . . . . .	53 5	78 2	6 16	4 48	10 64	5 496	4 192
Soil + H-ligno-proteininate . . . . .	59 4	86 2	1 12	9 52	10 64	7 011	5 707
Soil + Ca-ligno-proteininate. . . . .	76 7	112.1	0 98	10 08	11 06	6 927	5 623
Soil + casein. . . . .	293.5	399 3	80 36	29 40	109.76	1.435	0 131

amount of nitrogen liberated as nitrate in the soil, no doubt as a result of its consumption by the microorganisms decomposing the lignin. The  $\alpha$ -humus isolated from ordinary field soil, or the typical "humic acid," decomposed at about the same rate as the lignin, or also very slowly. However, in view of the fact that the C:N ratio of this complex has become stabilized, with a nitrogen content of a little over 3 per cent, typical of such preparations, the decomposition of the complex should logically be accompanied by a liberation of nitrogen in an available form. An increase in decomposition above the control soil, as measured by the liberation of additional 31.7 mgm. of carbon as CO<sub>2</sub> in 30 days, was accompanied by an increase of 1.68 mgm. in the liberation of nitrogen as ammonia.

The decomposition of the ligno-protein complexes is most interesting. The H-ligno-proteininate decomposed at about the same rate as the  $\alpha$ -humus of the soil, as shown both by the CO<sub>2</sub> evolution and the nitrogen liberation. The Ca-ligno-proteininate decomposed somewhat more rapidly. However, the rate

of decomposition of this complex presented a marked contrast to the decomposition of the 0.95 gm. of casein, containing exactly the same amount of protein. One cannot doubt here the fact that a stable complex between the lignin and the protein has been produced, and that this complex behaves, as far as its decomposition in the soil is concerned, as typical soil "humus." This is further substantiated by the extraction of the "humus" from the soil by means of an alkali solution and its precipitation by means of hydrochloric acid. The  $\alpha$ -humus used in this experiment was practically all recovered in the "soil humus" fraction; the same was exactly true of the synthesized ligno-protein complexes.

*Chemical nature of the ligno-protein complexes*

For the purpose of studying further the nature of the complex formed between casein, lignin, and various bases, especially for the purpose of establishing whether we are dealing here with a stable chemical complex or with a

TABLE 11

*Solubility of alkali lignin, ligno-proteins, and "soil humus" in cold and hot alcohol and in acetone*

PREPARATION*	MATERIAL SOLUBLE IN COLD ALCOHOL	MATERIAL SOLUBLE IN HOT ALCOHOL	TOTAL ALCOHOL- SOLUBLE	TOTAL NITROGEN IN ORIGINAL PREPA- RATION*	NITROGEN CONTENT OF ALCOHOL-SOLU- BLE PORTION	SOLUBLE IN HOT ACETONE	NITROGEN CONTENT OF ACETONE-SOLU- BLE PORTION
	per cent	per cent	per cent	mgm.	mgm	per cent	mgm.
Lignin.....	48 1	10 3	58 4	8 82	7.21	66 1	5 76
H-ligno-proteinates ...	38 6	10 7	49 3	63 00	31 01	49 1	11.04
Ca-ligno-proteinates....	1 2	1 2	2 4	59 64	....	0 3	....
$\alpha$ -humus from peat . . . . .	22 8	14 0	36 8	88 80	20 95	20 1	4 68

\* Three grams of air-dry material.

physical or absorption complex, several preparations were subjected to a detailed chemical analysis. This consisted in determining the composition of these preparations, their behavior to different solvents, their oxidation capacity, their reactivity with acetyl bromide, etc. Three-gram portions of several of the preparations were at first extracted with cold 95 per cent alcohol for 24 hours, followed by extraction under a reflux condenser with hot alcohol for 2 hours. The extracts were removed by filtration and evaporated in weighed dishes; a portion of the extract was then analyzed for total nitrogen. Similar extraction was made with hot acetone for 2 hours.

The results presented in table 11 point definitely to the formation of a stable complex between the lignin and the protein, similar in its nature to "soil humus." The lignin preparation was readily soluble in alcohol and in acetone; however, the ligno-protein complexes were less soluble, especially the calcium complex, which was practically insoluble in both acetone and alcohol. This may explain the commonly observed phenomenon that soil humus gives very

little alcohol-soluble material, while it is still present in the soil, but once it has been extracted by an alkali solution and precipitated by an acid, a large part of the humus is found to be alcohol soluble, as shown in the case of the  $\alpha$ -humus preparation. More important than this is the fact that, in the case of the H-complex, a large part of which is soluble in alcohol, the percentage of nitrogen in the soluble portion is the same as in the initial complex. This points to the chemical nature of the complex. Were the ligno-protein complex a mere physical mixture, one would expect that the lignin would become partly soluble in the alcohol, while the protein would remain insoluble; the fact that the alcohol extracted a proportional amount of the protein and of the lignin points to a certain chemical combination between the two.

TABLE 12  
*Oxidation of alkali lignin, ligno-proteins, and "soil humus" by 6 per cent  $H_2O_2$*

PREPARATION	MATERIAL OXIDIZED
	<i>per cent</i>
Lignin . . . . .	7 2
H-ligno-proteinate . . . . .	17 6
Ca-ligno-proteinate . . . . .	38 3
$\alpha$ -humus from peat . . . . .	75 8

TABLE 13  
*Oxidation of synthesized and natural "humus" complexes by 6 per cent  $H_2O_2$*

PREPARATION	RATIO OF LIGNIN TO CASEIN	AMOUNT OXIDIZED
		<i>per cent</i>
Lignin . . . . .	1:0	4 9
H-ligno-proteinate . . . . .	8:1	11 8
H-ligno-proteinate . . . . .	4:1	13 1
H-ligno-proteinate . . . . .	2:1	17 4
$\alpha$ -humus from mineral soil . . . . .	. .	28 2
$\alpha$ -humus from lowmoor peat . . . . .	. .	95 6
Electrodialyzed humus . . . . .	. .	46 1

In order to determine to what extent the ligno-protein complexes are readily oxidized with weak oxidizing agents, the various preparations were treated with hot 6 per cent  $H_2O_2$  (table 12). It should be recalled that this reagent has been recommended for determining the "humified" part of the soil organic matter. The results show that the ligno-protein complexes are "humified," since they are attacked readily by this reagent. The chemical nature of the preparation has a marked influence upon the extent of its oxidation; in this respect different "humus" preparations from soil behave similarly, as shown in table 13.

The older literature is full of references to determinations of "humus" by extracting the soil with ammoniacal solution, evaporating the extract, and

weighing the residue. This procedure, originally suggested in 1872 by Grandeau, giving "matière noire" or black matter, was later variously modified; it probably served more than any other procedure in confusing rather than in advancing our knowledge of the nature and origin of soil humus. Without the least consideration being given to the nature of the complexes extracted by this reagent, thousands of determinations of "humus" were made all over the world. In order to illustrate the bearing that this method may have upon the synthesized humus complexes, the various preparations were extracted for 24 hours with 4 per cent  $\text{NH}_4\text{OH}$  solution. The extracts were filtered and evaporated; the residues were dried and weighed (table 14). The results show that practically all the lignin, the H-ligno-proteinate and the  $\alpha$ -humus from peat were soluble in cold ammonium hydroxide solution, whereas only one-fifth of the Ca-complex was dissolved by this reagent. This is the reason why in all the determinations of the "humus" by the Grandeau method, it was always recommended to treat the soil first with hydrochloric acid, which results in

TABLE 14

*Solubility of lignin, ligno-proteins, and "soil humus" in cold ammonium hydroxide solution*

PREPARATION	SOLUBLE IN $\text{NH}_4\text{OH}$
	<i>per cent</i>
Lignin . . . . .	97.5
H-ligno-proteinate. . . . .	97 4
Ca-ligno-proteinate. . . . .	19 2
$\alpha$ -humus from peat . . . . .	97 4

the dissolution of the calcium or the substitution in the complex of hydrogen for the calcium.

It is well known that although proteins are readily hydrolyzed by hot dilute mineral acids, especially after prolonged boiling, the organic nitrogenous compounds of the soil are only partly hydrolyzed. This gave rise to the assumption that some of the nitrogen in the soil humus is not of a protein nature, but is present in the form of ring compounds, or in the form of "humin-nitrogen," whatever that may be. In order to compare further the synthesized complexes with soil humus, 3-gm. portions of the various preparations were treated with 100 cc. 5 per cent hydrochloric acid solution, at  $100^\circ\text{C}$ . for 2 hours; the nitrogen thus rendered soluble was then determined (table 15). The results reported show that whereas over 80 per cent of the nitrogen in the casein was brought into solution by dilute acid hydrolysis, only 20 to 33 per cent of the nitrogen was rendered soluble in the case of the ligno-proteinate and about 30 per cent in the case of the "soil humus." The artificial humus preparations behaved again in exactly the same manner as the natural soil humus.

These results seemed so important that a number of other preparations were treated with dilute hydrochloric acid (2 per cent), for 2 hours at  $100^\circ\text{C}$ . and

with hot 95 per cent alcohol (2 hours at 100°C.); these data were compared with the decomposition of the various preparations in soil. For this study, 3-gm. portions of the various preparations were added to 100-gm. portions of field soil; the cultures were incubated for 32 days and the CO<sub>2</sub> liberation was measured. At the end of the incubation period, the amount of nitrate produced was determined. The results (table 16) obtained fully confirm the previous observations.

TABLE 15  
*Influence of acid hydrolysis upon the ligno-proteins and "soil humus"*

PREPARATION	UNHYDROLYZED RESIDUE	PER CENT OF PROTEIN NITROGEN HYDROLYZED
	<i>per cent</i>	
Casein. ....	6 8	80.6
Lignin .....	94 3	3 2
H-ligno-proteinates .....	89 3	32.9
Ca-ligno-proteinates . . . .	80 3	20 2
α-humus from peat .....	83.0	29.9

TABLE 16  
*Influence of acid hydrolysis, treatment with hot alcohol, and microbial decomposition upon several ligno-protein preparations*

NATURE OF PREPARATION	NITRO- GEN CONTENT	HYDROLYSIS WITH HCl		SOLUBILITY IN 95 PER CENT ALCOHOL		DECOMPOSITION BY MICROORGANISMS IN SOIL	
		Per cent of total preparation hydro- lyzed	Per cent of total nitrogen hydro- lyzed	Per cent of total preparation brought into solution	Per cent of total nitrogen brought into solution	CO <sub>2</sub> liberated	Nitrate formed
	<i>per cent</i>					<i>mgm. C</i>	<i>mgm. N</i>
Lignin alone .....	0 12	2 0	45	13 6	.	44 1	1.3*
H-ligno-caseinate.....	2 68	11 2	32	41 8	33 4	46 1	3 2
H-ligno-albuminate. . . . .	3.01	12 8	35	47 0	14 0	40 6	2.5
Fe-ligno-caseinate.....	2 96	13.7	32	39 2	18 1	48 9	2 2
Fe-ligno-albuminate.....	2 91	6 7	16	....	..	56 6	2 5
α-humus from peat.....	2 94	23 7	23	..	.	..	..

\* Control soil liberated 37.5 mgm. carbon as CO<sub>2</sub> and 2.3 mgm. NO<sub>3</sub>-N.

Finally one crucial experiment may be reported here, namely, the behavior of the artificial humus preparations and of natural humus to the acetyl bromide reagent. Karrer and Boding-Wieger (12) found that acetyl-bromide dissolves all plant constituents, but does not act upon "humic bodies"; they suggested, therefore, the separation of humus from undecomposed plant materials by means of this reagent and its determination quantitatively. Groszkopf (9) and Springer (21) used this reagent extensively for the determination of the



"humus" content in soil and in peat. The fact that certain humus complexes, namely, the so-called "hymetomelanic acid" or the alcohol-soluble "humic acid," are oxidized by acetyl-bromide led Groszkopf to consider the latter as lignin-like in nature.

One-gram portions of the various preparations were treated with 50-cc. portions of acetyl-bromide at 50°C. for 8 hours. At the end of that period of time, the extracted material was filtered through Gooch crucibles, the residue was dried, washed with ether, dried again, and weighed (table 17). The results show that whereas lignin is nearly completely dissolved by acetyl-bromide, the  $\alpha$ -humus from peat is attacked only to a limited extent. The ligno-proteinates stand midway, the hydrogen complex being acted upon to the extent of nearly 90 per cent and the calcium complex to about 53 per cent. These results as well tend to show that as a result of interaction of the protein, lignin, and base, a new complex is formed which is more resistant to the action of acetyl-bromide than the original constituents; this complex tends to approach in this respect as well the humus of the soil.

TABLE 17

*The action of acetyl-bromide upon lignin, ligno-proteins, and "soil humus"*

PREPARATION	MATERIAL ATTACKED BY ACETYL-BROMIDE
	per cent
Lignin . . . . .	99 0
H-ligno-proteinate . . . . .	89 8
Ca-ligno-proteinate . . . . .	53 2
$\alpha$ -humus from peat . . . . .	21 4

#### DISCUSSION

The results of the experiments presented, as well as others which will be published in one of the following contributions to this subject, amply illustrate the fact that by the interaction of lignin and protein a complex is formed which possesses all the characteristic properties of the major fraction of the humus of the soil, namely, that part which is soluble in alkalies and is precipitated by acids, and which has commonly been referred to as "humic acid." The comparative characteristic properties of this complex with that of  $\alpha$ -humus or "humic acid" of the soil can be briefly summarized as follows:

PROPERTY OR REACTION	$\alpha$ -HUMUS OF SOIL OR PEAT	HUMUS-NUCLEUS OR LIGNO- PROTEINATE
Nitrogen content . . . . .	2-3 per cent	2-3 per cent
Combination with bases . . . . .	Combines readily	Combines readily
Solubility in dilute alkali solutions . .	Soluble	Soluble
Solubility in dilute mineral acids . . .	Insoluble	Insoluble
Oxidation by $H_2O_2$ . . . . .	Readily oxidized	Readily oxidized
Oxidation by acetyl bromide . . . . .	Only slightly oxidized	Not fully oxidized
Decomposition by microorganisms . . .	Attacked with difficulty	Attacked with difficulty
Influence on microbial processes . . .	Favorable	Favorable

Figure 3 gives a schematic presentation of the mechanism of humus formation, as a result of the decomposition of plant residues, on the basis of these and other investigations carried out in this laboratory. The assumption is thereby made that soil humus, or the total soil organic matter, consists of a humus-nucleus, resistant to decomposition, and accompanying substances, which consist of certain plant constituents and synthesized complexes undergoing decomposition. It is, of course, understood that the formation by condensation of a ligno-protein compound, or of a humus nucleus, can account for only a part of the humus in average soils. A number of chemical reactions in connection with the soil organic matter cannot be explained by the presence of these compounds alone. It is sufficient to mention the uronic acid com-

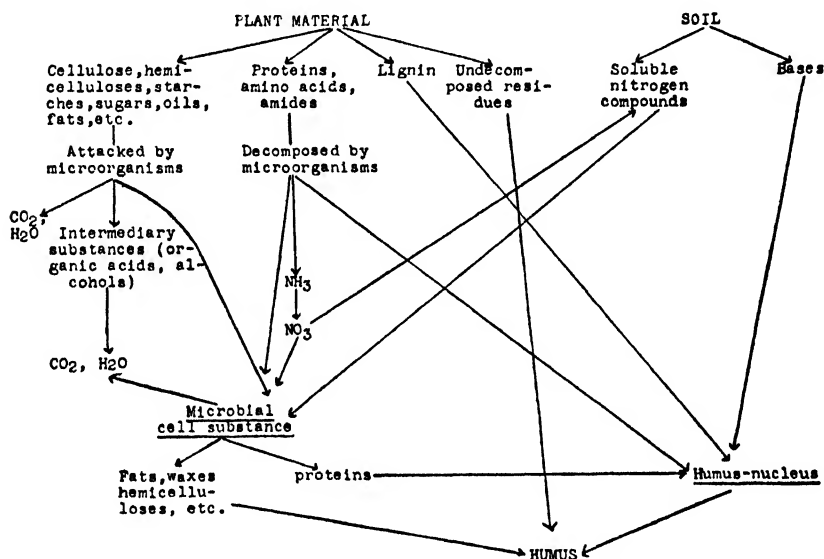
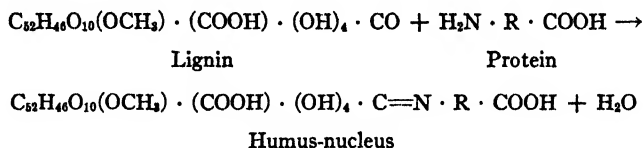


FIG. 3. SCHEMATIC REPRESENTATION OF THE MECHANISM OF HUMUS FORMATION IN THE DECOMPOSITION OF PLANT RESIDUES IN THE SOIL

plexes in soil and the presence of various aldehydes, acids, and alcohols (19, 20). Further, in view of the fact that proteins, varying considerably in chemical composition, enter the soil in the plant and animal residues or are formed there through the activities of the microorganisms, while lignins may also vary considerably in chemical composition, the nature of the ligno-protein complexes formed under different conditions will vary. The nature of the base seems to be also of considerable importance in modifying the chemical nature and reactivity of the complexes. Although the results reported here deal largely with the reactions of the hydrogen and calcium complexes, the information which is now being accumulated and which will be reported later on the behavior of the iron and aluminum compounds, shows that these approach, in their characteristics, the soil humus even more than the other two complexes.

As to the chemistry of the reaction of condensation involved between the protein and the lignin, several possibilities suggest themselves. These are based upon the fact that lignin is known to possess carboxyl groups, hydroxyl groups, carbonyl groups, methoxyl groups, in addition to the cyclic rings, or the ring skeleton (7). If one assumes that the COOH group of the lignin interacts with the NH<sub>2</sub> group of the protein, a salt formation will take place, even in an alkaline solution; a reaction of this type takes place in the process of tanning. The possibility of interaction between a phenolic OH group and the NH<sub>2</sub> group would meet with the objection that, aside from the improbability of such a reaction taking place, the complex itself could be readily hydrolyzed. The assumption of a reaction between the NH<sub>2</sub> group of the protein molecule with a carbonyl group, of a ketonic or an aldehydic nature, to form a compound of the nature of a Schiff's base, offers a fair degree of probability.



This complex would be highly stable and would possess a high base exchange capacity, a phenomenon characteristic of the humus-nucleus. However, since the combining capacity of proteins with bases and acids cannot be fully accounted for by the free carboxyl or amino groups of the protein molecule and since the CO·HN groups in the interior of the molecule are capable of combination, giving compounds not readily subject to hydrolytic dissociation, various other combinations between proteins and lignins suggest themselves.

#### SUMMARY

1. Studies are reported concerning the influence of lignin upon the decomposition of proteins by pure and mixed cultures of microorganisms.
2. Lignin was found to exercise a depressive effect upon the decomposition of proteins; this depression is not due to any toxic action of the lignin, but is a result of the interaction of the lignin with the protein, which makes the latter more resistant to attack by microorganisms.
3. A resistant complex consisting of lignin and protein, two of the important constituents of plant, animal, and microbial residues, has been synthesized in the laboratory. This complex is similar in physical appearance and possesses the various chemical, physico-chemical and biological properties characteristic of the major portion of the soil organic matter, which is usually referred to as "humus" or "humic acid."
4. The ligno-protein complexes will combine with various bases, such as calcium, magnesium, iron, and aluminum, in a manner similar to the combination between soil humus and these bases.
5. In view of the fact that the major portion of the soil humus is made up of

lignin complexes, or their derivatives, and of protein complexes, it is suggested that the soil humus consists largely of these two compounds combined to form new chemical complexes.

6. The synthesized ligno-protein complex will be referred to as the "humus-nucleus," in contradistinction to the term "humus," which is reserved for the soil organic matter as a whole; the term "synthetic humus" cannot be used, since it has been commonly employed to designate the dark colored substances obtained on treatment of sugar with mineral acids, or by the oxidation of phenols.

7. The synthesis of the humus-nucleus enables one to carry out studies of the physical, chemical, and biological behavior of soil humus and its relation to the inorganic soil constituents, without having to use the complex humus of the soil itself, since this humus varies considerably in chemical composition, depending on the soil from which it is obtained and upon the method of isolation.

8. The formation of a humus-nucleus complex establishes definitely the relation between the organic nitrogenous substances and the non-nitrogenous substances of the soil humus; this is responsible for the resistance of the soil nitrogen to rapid decomposition by microorganisms. The formation of such a complex also suggests evidence to explain the more or less constant carbon-nitrogen ratio which exists in mineral soils.

9. The synthesis of the humus-nucleus points to an explanation for the relation between the organic complexes in the soil and the inorganic soil constituents, especially the soil bases. At a pH less than 4.8, the ligno-protein complex is largely saturated with hydrogen, iron, and aluminum. The higher the pH value, the greater will be the replacement of the H-ion by Ca and Mg ions and finally, at a pH of 9.0 or above, by Na-ions. At a reaction of pH less than 4.8, most of the complex exists as a hydrogen-ligno-proteinate and also as Fe- and Al-ligno-proteinates; at a pH of 4.8 to 9.0, it exists largely as Ca- and Mg-ligno-proteinates, and probably also as Fe- and Al-ligno-proteinates; and at pH above 9.0, especially at 9.6-10.0, it exists largely as a sodium-ligno-proteinate.

10. The exact chemical nature of the complex still remains to be studied; its nature varies with the nature of the lignin and proteins used, relative concentration of these two substances, reaction at which precipitation takes place, relative concentration and nature of bases, degree of drying, and a number of other factors.

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# CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: II. THE INFLUENCE OF "SYNTHESIZED" HUMUS COMPOUNDS AND OF "NATURAL HUMUS" UPON SOIL MICROBIOLOGICAL PROCESSES<sup>1</sup>

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The rôle of humus in soil processes has frequently been ascribed to its beneficial action upon the growth and activities of soil microorganisms. Among these, the influence of humus upon the growth of *Azotobacter* and other nitrogen-fixing bacteria has received particular attention (3). It was believed that this favorable effect of humus is due to its colloidal properties, but recent information tends to prove that this is due, both as regards plant growth (4) and the development of *Azotobacter* (1, 5), to the iron content of the humus.

Among the other phenomena concerned with the influence of humus upon microbial development, the extent to which humus can be used by microorganisms has also received considerable attention. Although the ability of bacteria and fungi to attack "humic acids" as sources of carbon has been denied, the utilization of these acids as sources of nitrogen was considered possible (6).

In order to study the influence that synthesized humus complexes may have upon microbiological activities, whether toxic, favorable, or neutral, as compared with that of natural soil humus, or organic compounds found in soil and peat, several microbiological processes, characteristic of the soil, were investigated.

The first experiment deals with the influence of humus upon the decomposition of glucose by microorganisms. The following medium was prepared:

	gm.		gm.
Glucose.....	20	NaCl.....	0.1
NaNO <sub>3</sub> .....	2	FeSO <sub>4</sub> .....	0.02
K <sub>2</sub> HPO <sub>4</sub> .....	1	Distilled water.....	1.000
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	0.5		

This medium was distributed into flasks, some of which received the ligno-protein complexes and others did not. Another medium of similar composition, but free from nitrate, was also prepared, so as to determine to what extent the

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nitrogen in the ligno-protein complexes can be used by the organisms decomposing the glucose. The media were sterilized, inoculated with soil infusion, and incubated for 6 days. At the end of that period, the remaining glucose in the cultures was determined. The results presented in table 1 show that

TABLE 1

*Influence of lignin and ligno-proteinates upon the decomposition of glucose by microorganisms*

TREATMENT OF CULTURE	NITROGEN ADDED	GLUCOSE LEFT	GLUCOSE DECOMPOSED
		mgm.	mgm.
Glucose alone, uninoculated*	—	974	0
Glucose alone, uninoculated	+	974	0
Glucose alone, inoculated	—	906	68
Glucose alone, inoculated	+	615	359
Glucose + lignin†	—	900	74
Glucose + lignin	+	773	201
Glucose + H-ligno-proteinatet	—	917	57
Glucose + H-ligno-proteinatet	+	643	331
Glucose + Ca-ligno-proteinatet	—	720	254
Glucose + Ca-ligno-proteinatet	+	607	367

\* One gram of glucose in addition to the mineral nutrients.

† Three grams of lignin used per flask.

‡ One gram of the ligno-proteinates used.

TABLE 2

*Influence of lignin, ligno-proteinates, and "soil humus" upon the decomposition of cellulose by microorganisms*

SUBSTANCES USED	NITROGEN USED AS NITRATE	TOTAL CO <sub>2</sub> LIBERATED	CELLU- LOSE DECOM- POSED	NITRATE CON- SUMED	RATIO OF CELLULOSE DECOMPOSED TO NITROGEN CONSUMED
		mgm. C.	mgm.	mgm. N	
Control medium*	0	9.0	47.7	.....	....
Ca-ligno-proteinatet, 1 gm.	0	25.9	.....	.....	....
H-ligno-proteinatet, 1 gm.	0	18.5	.....	.....	....
α-humus, 1 gm.	0	17.7	.....	.....	....
Control medium	+	132.7	438.5	13.78	31.8
Lignin, 2 gm.	+	185.0	522.0	16.12	32.4
Ca-ligno-proteinatet, 1 gm.	+	185.3	552.3	16.56	33.4
H-ligno-proteinatet, 1 gm.	+	159.0	480.2	14.76	32.5
α-humus, 1 gm.	+	159.4	468.7	14.48	32.3

\* One-gram portions of cellulose used in each culture.

lignin alone had a somewhat depressing effect, in the presence of available nitrogen in the culture, upon the decomposition of the glucose. The H-ligno protein complex had only a very slight depressing effect, both in the presence and absence of added nitrogen, probably due to the acidity of the complex, since the medium was poorly buffered. However, the calcium-ligno-protein

complex had a marked favorable effect, especially in the absence of added inorganic nitrogen. Whether this is due to the fact that the microorganisms decomposing the glucose could utilize the complex as a source of nitrogen, or whether it is due to the favorable effect of the complex upon the development of the bacteria, especially the nitrogen-fixing organisms, remains to be determined.

The following experiment deals with the influence of ligno-protein complexes, as compared with that of lignin, on the one hand, and of soil humus ( $\alpha$ -humus), on the other, upon the decomposition of cellulose by microorganisms. This experiment will tend to establish whether the synthesized humus complexes have (a) an injurious effect upon an important microbiological process, or a

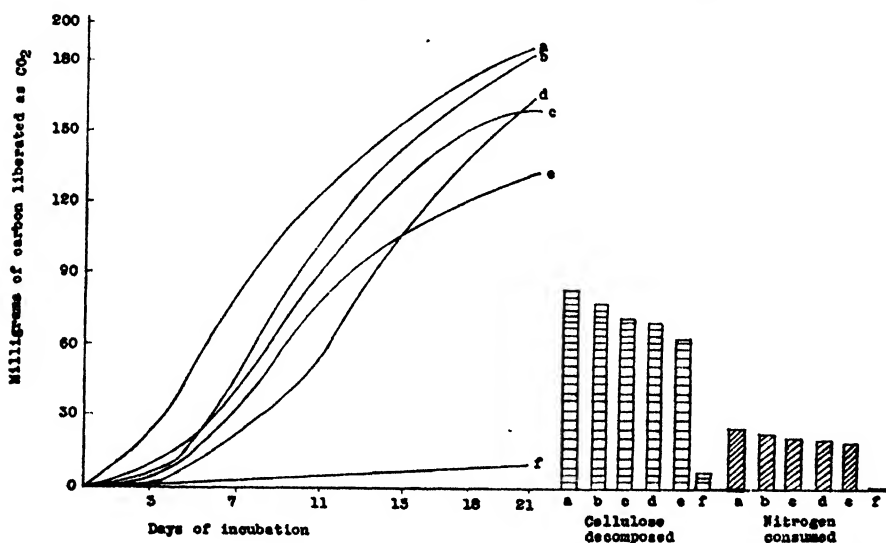


FIG. 1. THE INFLUENCE OF LIGNIN, LIGNO-PROTEIN COMPLEXES, AND SOIL "HUMUS" UPON THE DECOMPOSITION OF CELLULOSE BY MICROORGANISMS IN SAND MEDIA, AS MEASURED BY EVOLUTION OF CO<sub>2</sub>

a, Ca-ligno-proteinate; b, alkali lignin; c, H-ligno-proteinate; d, soil "humus"; e, cellulose alone; f, cellulose without inorganic nitrogen.

favorable effect, or no effect at all, and (b) whether the nitrogen of the protein in the complex can be used as a source of nitrogen by the cellulose-decomposing organisms.

The experiment was carried out in a sand medium. In each of several long-necked flasks 100 gm. of washed sand and 1 gm. of ground air-dry cellulose were placed; the different humus preparations were then added and well mixed with the sand and the cellulose. To each flask 20 cc. of distilled water containing the necessary minerals was added. Some of the flasks received 200 mgm. of NaNO<sub>3</sub> and others received no inorganic nitrogen. The flasks were inoculated with a soil infusion and incubated for 21 days. The cultures were

aerated daily by passing a current of air above the medium, and the  $\text{CO}_2$  was measured. At the end of the incubation period, the residual cellulose in the cultures was determined. In those cultures, which received additional inorganic nitrogen in the form of sodium nitrate, the residual nitrate was also determined. The results are reported in table 2 and in figure 1.

The results show that the ligno-protein complexes, as well as the soil humus ( $\alpha$ -humus), cannot be used by the cellulose-decomposing bacteria as sources of nitrogen. Only traces of cellulose were decomposed in the absence of inorganic nitrogen, and in the presence of the organic nitrogenous complexes; the natural and synthesized humus complexes behaved practically alike. However, these complexes, as well as the lignin itself, have a decidedly beneficial effect upon the process of cellulose decomposition by microorganisms; this effect is not due to the utilization of these compounds as a source of carbon or nitrogen, as shown by the fact that the cellulose-nitrogen ratio was not modified,

TABLE 3

*Influence of lignin and of ligno-proteinates upon the decomposition of Trichoderma mycelium and casein by microorganisms*

MATERIAL ADDED	TOTAL $\text{CO}_2$ LIBERATED	$\text{NH}_3$ LIBERATED	$\alpha$ -HUMUS FOUND IN MEDIUM	NITROGEN IN $\alpha$ -HUMUS
	mgm. C	mgm. N	gm.	mgm.
Sand medium alone.....	1.7	0.56	0.003	....
Fungus mycelium, 1 gm.....	105.2	6.02	0.047	4.62
Fungus mycelium + 2 gm. lignin .....	78.9	2.38	1.554	19.18
Fungus mycelium + 1 gm. H-ligno-proteinat.....	81.6	4.34	0.771	24.22*
Fungus mycelium + 1 gm. Ca-ligno-proteinat.....	119.7	7.70	0.706	23.66†
Casein, 0.5 gm.....	105.5	53.20	0.006	2.80
Casein + 2.5 gm. lignin.....	74.7	38.08	2.000	18.48

\* Original nitrogen in complex = 22.6 gm.

† Original nitrogen in complex = 22.8 gm.

but is due to some other factor. The Ca-ligno-proteinat exerted the most favorable action. Whether this is due to the colloidal properties of the organic complexes, to their buffering properties, or to some other phenomenon still remains to be determined. The interesting point to note in this connection is that the synthesized H-ligno-proteinat had exactly the same effect upon cellulose decomposition as the "humus" or "humic acid" of the soil, which is also largely a hydrogen complex.

It is of considerable interest to determine the influence of lignin and of the artificial humus complexes upon the decomposition of organic nitrogenous compounds, not only in the state of free protein but also in the form of complex substances, of a nature similar to those that one would expect to find in the soil. For this purpose, the mass of growth of the fungus *Trichoderma*, grown on synthetic liquid media, and comprising both mycelium and spores, was employed. This material was selected for two reasons: (a) this organism is a common, cellulose-decomposing soil fungus, and it was found to synthesize

considerable cell substance in the soil; (b) this cell substance has a fairly high protein content (6.4 per cent nitrogen) and one would expect that a part of its nitrogen would be liberated as ammonia in the process of its decomposition. The same medium was employed as in the previous experiment, except that 1-gm. portions of fungous growth, instead of the cellulose, were used with no additional inorganic nitrogen. At the end of the incubation period (15 days), the ammonia nitrogen was determined in an aliquot portion of the residue, while the remaining portion was extracted with 4 per cent NaOH solution; the extract was acidified with HCl; the precipitate was filtered, washed, dried

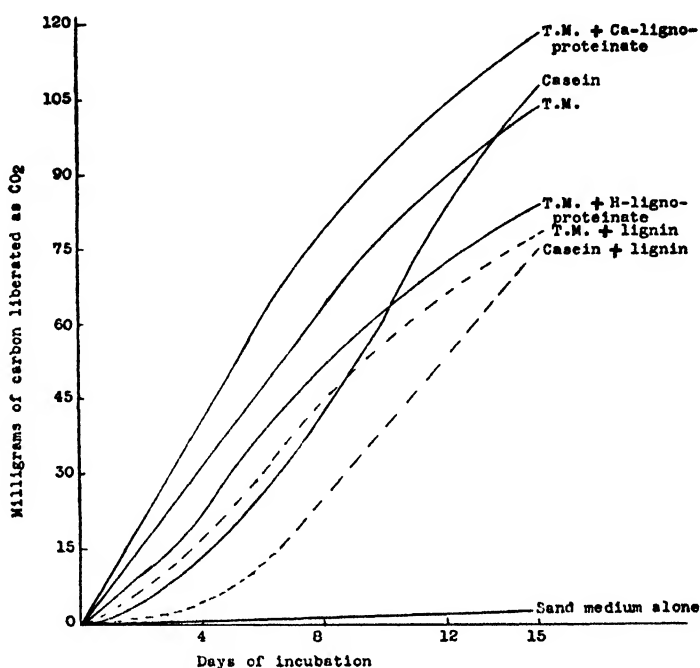


FIG. 2. INFLUENCE OF LIGNIN AND LIGNO-PROTEINS UPON THE DECOMPOSITION OF TRICHO-DERMA MYCELIUM (T. M.) AND CASEIN IN SAND MEDIUM, AS MEASURED BY THE EVOLUTION OF  $\text{CO}_2$

weighed, and analyzed for total nitrogen. The results are given in table 3 and in figure 2.

Lignin alone repressed the decomposition of the fungous mycelium, as shown by the reduction in the evolution of  $\text{CO}_2$ . The previous experiment would lead us to conclude that this reduction is due to a depression in the decomposition of the proteins and not of the carbohydrates. That this was actually the case is proved by the reduction in the ammonia liberated. The ratio between the reduction in the carbon liberated as  $\text{CO}_2$  ( $105.2 - 78.9 = 26.3$ ) and the reduction in the nitrogen liberated as ammonia ( $6.02 - 2.38 = 3.64$ ), was found to be 7.2. The ratio of the carbon to nitrogen in the mycelium is  $\frac{50}{6.4} =$

7.8. A similar depressing effect of the lignin was observed in the case of the casein. Here the ratio of C/N reduction is 2.0. The ratio of C/N in casein itself is about 3.0. The addition of the ligno-protein complexes produced a somewhat depressing effect in the case of the acid complex and a favorable effect for the calcium complex, with a corresponding liberation of the nitrogen as ammonia. In other words, the two ligno-protein complexes which are saturated with protein brought about no tying up of the protein. This is confirmed by the nitrogen content of the  $\alpha$ -humus fraction of the residual material. Whereas the lignin tied up nearly 14.5 mgm. of nitrogen as protein from the *Trichoderma* mycelium and somewhat more from the casein, the ligno-protein complexes did not tie up any more protein than they have already contained.

These results not only prove the formation of a ligno-protein complex in the process of decomposition of protein-containing material in the soil, in the

TABLE 4  
*Influence of lignin and humus preparations upon the fixation of nitrogen by Azotobacter vinelandii*

SUBSTANCES USED	INOCULATED WITH AZOTOBACTER	NITROGEN CONTENT	NITROGEN FIXED
		mgm.	mgm.
Control medium .....	—	0 35	....
Control medium .....	+	4 27	3 92
Lignin + CaCO <sub>3</sub> .....	—	1.20	. . .
Lignin + CaCO <sub>3</sub> .....	+	4 56	3 36
$\alpha$ -fraction + CaCO <sub>3</sub> .....	—	9.80	.
$\alpha$ -fraction + CaCO <sub>3</sub> .....	+	12 46	2 66
Ca-ligno-proteinate .....	—	10.22	. . .
H-ligno-proteinate + CaCO <sub>3</sub> .....	+	15 62	5 40
Ca-ligno-proteinate .....	+	15.54	5.32

presence of lignin, but they also demonstrate that the tendency is toward the establishment of a complex between lignin and protein of a definite chemical composition. Once such a complex is formed, there will be no further protein absorption or removal of the protein from active decomposition by micro-organisms. These phenomena have a highly important bearing upon the rapidity and amount of liberation of nitrogen in an available form, in the decomposition of plant and animal residues in the soil.

In the following experiments, the influence of the humus complexes synthesized in the laboratory, as compared with that of lignin and  $\alpha$ -humus from soil, upon the fixation of nitrogen by *Azotobacter vinelandii* was determined. In a series of flasks were placed 100-cc. portions of 1.5 per cent mannitol solution, containing the necessary minerals, but no combined nitrogen, and adjusted to pH 8.0. One-half gram portions of the several preparations were added to the various flasks. These were then sterilized for 15 minutes at 15

pounds pressure, inoculated with a pure culture of *Azotobacter vinelandii*, and incubated for 21 days. The results (table 4) show that the lignin and the  $\alpha$ -fraction of the soil had, under these experimental conditions, a somewhat depressive effect upon the fixation of nitrogen by the organism, probably due to their acid reaction, whereas the ligno-protein complexes had a decidedly beneficial effect.

In order to determine the nature of the stimulating effect of the synthesized humus complexes upon the fixation of nitrogen by *Azotobacter*, the last experiment was repeated under somewhat different conditions. In a series of 500-cc. flasks were placed 150-cc. portions of Ashby's medium. These flasks received varying quantities of calcium and iron ligno-proteinates, as well as of soil "humus" and of lignin. It has been shown by various investigators (3) that the addition of humus to pure cultures of *Azotobacter* increases considerably the

TABLE 5

*Influence of lignin, ligno-proteinates, and "soil humus" upon nitrogen-fixation by Azotobacter vinelandii*

TREATMENT	INOCULATED WITH AZOTOBACTER	NITROGEN CONTENT OF CULTURE	NITROGEN FIXED
		mgm.	mgm.
Control medium . . . . .	—	0.50	....
Control medium . . . . .	+	4.20	3.70
Lignin, 500 mgm. . . . .	—	1.20	....
Lignin, 500 mgm. . . . .	+	3.5	2.30
Ca-ligno-proteinates, 500 mgm. . . . .	—	9.80	....
Ca-ligno-proteinates, 50 mgm. . . . .	+	4.95	3.50
Ca-ligno-proteinates, 500 mgm. . . . .	+	13.20	3.40
Fe-ligno-proteinates, 500 mgm. . . . .	—	13.90	....
Fe-ligno-proteinates, 50 mgm. . . . .	+	7.70	5.85
Fe-ligno-proteinates, 500 mgm. . . . .	+	19.45	5.55
$\alpha$ -humus, 500 mgm. . . . .	—	15.40	...
$\alpha$ -humus, 500 mgm. . . . .	+	15.60	0.20

nitrogen-fixing capacity of this organism. Humus obtained from various sources was found to vary in its effect; humus prepared from sugars by boiling with acids has no such effect; on boiling humus with acid, the activating substance seems to be removed. The humus is not used by the organism either as a source of carbon or nitrogen. Burk et al. (1) have recently established the fact that the favorable effect of the humus upon nitrogen-fixation is due to its iron content.

The results presented in table 5 show that iron-ligno-proteinates had a decidedly favorable effect upon the fixation of nitrogen by the organism, but the Ca complexes, as well as the "natural soil humus," had no such effect. This "soil humus" was prepared by extracting soil with 4 per cent NaOH solution, acidifying the extract, boiling, filtering, washing, and drying; this "humus" had practically no favorable effect upon the growth of the organism above the

control and reduced considerably the fixation of nitrogen. Without attempting to analyze the nature of the action of the humus complexes upon nitrogen fixation by *Azotobacter*, it is sufficient to say that synthesized humus complexes, provided they contain iron, have an exactly similar favorable effect upon the process of fixation as has been reported for natural humus preparations which contain iron.

Samples of these synthetic preparations were submitted to Dr. D. Burk and his associates in the U. S. Department of Agriculture Bureau of Chemistry and Soils. The influence of these preparations was tested by them upon the growth of *Azotobacter* and *Rhizobium*, as determined by the Warburg technique, by measuring respiration, and by the relative turbidity; the synthetic preparations were compared with the action of natural humus preparations. The iron-ligno-proteinate was reported to compare quite favorably with "natural humic acids" containing iron, with respect to both induction period and amount of growth stimulation ultimately produced, and interestingly enough, in iron content.

#### DISCUSSION

The results presented in this paper contribute further information to the similarity in behavior between the "humus complexes" synthesized in the laboratory from lignin and protein and natural "humus complexes" isolated from soil and from peat. These results also show that in the process of decomposition of microbial cell substance, some of the protein constituents interact with the lignin to give rise to humus complexes. This tends to throw further light upon the rôle of soil microorganisms in synthesizing new complexes which contribute to the soil humus, either directly as the constituents of their cell substance, or indirectly, namely, by the interaction of some of the constituents of the microbial cell substance with some of the resistant constituents of the plant residues, especially the lignins.

#### SUMMARY

1. Results are reported on the influence of synthesized humus, or the humus-nucleus, upon certain important microbiological processes.
2. The synthesized humus complexes were found to have a favorable effect upon the decomposition of glucose by a mixed soil microbial population, especially in the absence of an added source of combined nitrogen.
3. Synthesized humus complexes as well as "natural soil humus" could not be used as sources of nitrogen by cellulose-decomposing microorganisms; however, they had a decidedly beneficial effect upon the decomposition of cellulose, in the presence of available combined nitrogen. The ratio between the cellulose decomposed and the nitrogen consumed by the microorganisms decomposing the cellulose was not modified by the presence of humus complexes.
4. Lignin was found to have no injurious effect upon cellulose decomposition; the effect was rather beneficial. This adds further weight to the idea

that the injurious effect of lignin upon protein decomposition is not due to any direct injury, but is indirect, namely, due to the formation with the protein of a complex, which is resistant to further decomposition by microorganisms, and which contributes to the formation of soil humus.

5. The decomposition of fungous mycelium was studied, in the absence and in the presence of lignin and ligno-protein complexes. Lignin was found to have an injurious effect upon this process, as measured by  $\text{CO}_2$  liberation and  $\text{NH}_3$  accumulation; a large part of the nitrogen of the mycelium could be isolated, at the end of the experiment in the form of a ligno-protein complex. The same was true of the influence of lignin upon the decomposition of casein.

6. Calcium-ligno-proteinate has a favorable effect upon the decomposition of the fungous mycelium, as measured by both  $\text{CO}_2$  evolution and ammonia accumulation. The hydrogen-ligno-proteinate, however, had a slightly injurious effect, possibly due to its acid reaction.

7. The synthesized humus complexes containing iron had a highly beneficial effect upon the fixation of nitrogen by *Azotobacter*.

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## BOOK REVIEW

*Sulphur Bacteria. A Monograph.* By DAVID ELLIS, Professor of Bacteriology, The Royal Technical College, Glasgow. Longmans, Green and Co., London, New York, 1932. Pp. IX + 261, figs. 66. Price, \$7.50.

This is the first book in English to be added to the numerous treatises of these interesting autotrophic microorganisms. It is composed of 13 chapters, which are devoted principally to the following subjects: sources of hydrogen sulfide; metabolism, culture, classification, and description of the sulfur bacteria; influence of light on the organisms; the nature of the pigments of the organisms.

The author's objective, to develop " . . . the present position of our knowledge of the sulphur bacteria," seems to have been incompletely attained. It is unfortunate that the book appeared at such a time as to miss the observations of van Niel (*Arch. Mikrobiol.*, 3 (1931): 1-112) which elucidate numerous previously confused features of the morphology and biochemistry of the purple and green sulfur bacteria.

Although little can be claimed for originality or completeness in the work, much that is interesting and informative can be derived by the discerning reader. However, certain defects appear which greatly detract from the value of the volume. These defects appear prominently in the biochemical discussions and start with the opening sentence: "The term Sulphur Bacteria is usually applied to the members of the group which have sulphur globules in their cells." This idea is retained throughout the work (*see* p. 84, 130, 220, 226) and the author excludes from the category of sulfur bacteria, those which are small rod-shaped forms generally classified among the Eubacteriales. Incidentally, this eliminates the sulfur bacteria which have received the most complete biochemical study at the present time. He appears to be unaware of the fact that the sulfur bacteria are not significant as physiological entities because of the presence of sulfur within the cells, but because of their ability to utilize the energy liberated in the oxidation of sulfur or incompletely oxidized compounds of sulfur. The products of oxidation are incidental and differ with the various organisms. It has further been shown by van Niel that storage of sulfur globules within the cells is dependent entirely upon the size of cells and has no other taxonomic, morphological, or biochemical significance.

His interpretation of the transfer of energy in the nutrition of the organisms is very confused and inconsistent with known facts, to judge only by the following typical examples: " . . . this substance ( $H_2S$ ) is not the source of either the food or the energy of the sulphur bacteria" (p. 47) and " . . .

the change from sulphide to sulphur does not result in a liberation of energy" (p. 46). The impression is also given that the sulfur bacteria are unable to utilize the energy derived from oxidation of sulfur to sulfate (p. 33, 38).

Many misstatements are rather apparent. The following may suffice to illustrate the point. "The sulphate may be reduced to elementary sulphur if the supply of oxygen be scanty" (p. 8). (No direct microbiological change of this nature is known to the reviewer.) "No experimental evidence for the existence of this ferment (Philothion) has been supplied" (p. 24). (See however the glutathione of Hopkins and associates.) ". . . the organism (*Thiobacillus denitrificans*) is one of the so-called obligate anaerobes" (p. 49). (This organism also develops aerobically.) "The sulphur compounds must be regarded as fermentable substances" (p. 50). (This is an unwarranted use of the term "fermentable substances.") Regarding *Thiobacillus thiooxidans*, he states, "The culture medium is acidified by its development, and the amount of thiosulfate increases steadily with the growth of this species." (p. 223). (The term "thiosulfate" is wrong and should be "sulfate.") "All of the chromoparus sulphur bacteria are purple" (p. 232). (There are also green forms.)

Morphology and classification occupy prominent portions of the volume. Although such information may be of aid in further studies, its value is limited in view of the great pleomorphism noted in those species which have received detailed study in the past. Without justification, the author appropriates the generic name *Thiobacillus* for one of the groups of the higher sulfur bacteria, although its use has been well established for a group of the sulfur bacteria included among the Eubacteriales, which the author naively would dismiss from the sulfur bacteria.

It is to be regretted that this group of organisms which is so fascinating from the biochemical viewpoint and which plays rôles of considerable economic importance in nature, should not have received more accurate and detailed consideration from the biochemical standpoint.

ROBERT L. STARKEY.

# THE APPLICABILITY OF THE AZOTOBACTER (PLACQUE) METHOD FOR DETERMINING THE FERTILITY REQUIREMENTS OF ARIZONA SOILS<sup>1</sup>

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The soils of Arizona are characterized by extremely low nitrogen and organic matter contents, by relatively large amounts of total phosphorus and potassium, and by varying amounts of calcium carbonate. Practically all of the soils now under cultivation are calcareous—the quantities of  $\text{CaCO}_3$  ranging from 2 to 20 per cent. During the past 4 years the author has analyzed approximately 4,000 soil samples which come from all parts of the state. Only two samples showed pH values lower than neutrality; the average was approximately pH 7.8.

Comparatively speaking, agriculture in Arizona is still in its infancy. Although Arizona ranks fifth among the 48 states in area (113,956 square miles) less than 1 per cent of this area is used at present for tilled agriculture. With the further development of water resources, the number of acres used for agricultural purposes will increase.

Few fertilizer experiments have been conducted. Field practices have shown that nitrogen fertilization is often helpful. It is generally assumed that the soil contains an abundance of potassium. This would be expected, since most of the soils are of granitic origin, and orthoclase feldspar would be the source of potassium. There is no evidence, to date, that Arizona soils need potash fertilizers.

Although these soils contain relatively large amounts of phosphorus, the amounts of available phosphorus are small, because of a rapid reversion to insoluble forms. This question of availability has been discussed by Breazeale and Burgess (1) and by McGeorge and Breazeale (11, 12), and will be given further attention in a series of technical bulletins to be published by the Arizona Agricultural Experiment Station. The only systematic field study of the effects of phosphorus fertilization was made by Crider (5), who showed that lettuce responded to additions of phosphorus. Recent field tests have shown that alfalfa and truck crops often respond to phosphate fertilization.

In the past, fertilizer practice has been limited principally to the application of nitrogen, chiefly in the form of barnyard or green manure. More progressive farmers, however, are commencing to add phosphorus to their soils.

<sup>1</sup> Contribution from the department of agricultural chemistry, Arizona Agricultural Experiment Station.

Consequently there is an apparent need for a method of determining the fertility requirements of these soils. The chemical methods in present use are of little value in determining the availability of fertilizers in these soils. McGeorge (10), in this laboratory, has obtained, to a certain extent, correlation between rate of liberation of phosphate ions during electrodialysis and phosphate availability as determined by the growth of plants. This method has already been discussed (10). The determination of replaceable potassium gives a fair index as to the potassium needs of soils.

The limited value of chemical methods of determining fertilizer requirements of soils has served to stimulate research in biological methods. Christensen (2) in 1907, suggested the use of *Azotobacter* in mannite solutions, with and without lime, as a means of determining the lime requirement of soils. He later (3, 4) extended this method to test for phosphorus deficiencies in soils, by inoculating a mannite solution of varying phosphorus content with soil. In 1910, Dzierzbicki (6) showed that soils deficient in available lime, phosphoric acid, or potash contained very small numbers of nitrogen-fixing organisms, particularly *Azotobacter*, and that in some cases these organisms were absent. Stoklasa in 1911 (18) claimed that there is a direct relationship between nitrogen fixed by *Azotobacter* and the amount of phosphorus assimilated. Niklewski in 1912 (13) presented data showing that an approximation of available phosphorus may be obtained by adding a given amount of soil to a mannite solution free from phosphorus, inoculating with a pure culture of *Azotobacter*, and then determining the amount of nitrogen fixed at the end of a definite incubation period. Little progress was made in the development of these methods until Winogradsky (20, 21, 22) commenced his studies of nitrogen-fixing organisms. From these studies the spontaneous culture method was developed. A source of energy (usually starch or mannite) is added to a given amount of soil, water is added, and the soil is worked into a thick paste. A quantity of the paste is placed in a Petri dish, the surface smoothed by means of a glass slide, and the plates are then incubated. In approximately 48 hours, *Azotobacter* colonies commence to appear upon the surface of the soil. Winogradsky found that by the addition of lime, and some soluble phosphate, it was possible to estimate the phosphorus or lime requirements of soils. A comparison of these tests with crop yields gave a close correlation.

Sackett (16) has applied this method to Colorado soils with marked success and recommends its extensive adoption as a routine procedure in determining the phosphorus, potash, and lime requirements of the soils of that state. He has given the method such wide circulation in farm journals, by radio, and before scientific meetings, that it has aroused the interest of Arizona farmers. Although the test, as described by Walker (19), was used in this laboratory in 1929, the results were not sufficiently encouraging to warrant further use. In view of the publicity the method has received recently, a further investigation was made in order to determine its value for determining the mineral deficiencies of Arizona soils.

## EXPERIMENTAL

The samples used were taken under aseptic conditions as far as possible, and were stored in sterile containers. These samples represent the principal agricultural sections of the state. A few soils had been used by McGeorge and Breazeale (11, 12) in some phosphate availability studies. These soils were used here because data were available concerning their phosphate contents and the probable phosphate availability, as determined by the electrodialysis method and by pot experiments with plants.

The technique used was that described by Walker (19). Sackett used both sodium and potassium phosphate, in order to estimate both potassium and phosphorus deficiencies, whereas Walker used only sodium phosphate. Since all of the soils examined were calcareous, calcium carbonate was not added. One hundred gram portions of the soil to be tested were weighed into mixing bowls and treated in the following manner:

1. Check—5 gm. potato starch
2. 5 gm. potato starch + 0.6 gm.  $\text{Na}_2\text{HPO}_4$
3. 5 gm. potato starch + 0.3 gm.  $\text{K}_2\text{HPO}_4$

The soil and added materials were then thoroughly mixed, and sufficient water was added to make a thick paste. This was then transferred to two halves of 50-mm. Petri dishes, and the surface smoothed by means of a moist spatula. The plates were placed in moist chamber culture dishes and incubated at 30°C. The plates were examined daily. In most soils colonies appeared within 24 to 48 hours; the maximum number appeared within 72 hours. Incubation was continued for 7 days, in order to eliminate the possibility of slow growers, which might otherwise be overlooked. The number of colonies per placque were counted at the end of 72 hours.

The first tests were made upon soils that had been used previously in some nitrogen fixation studies, and were known to contain *Azotobacter*. Since a relatively large number did not develop colonies on the plaques, in order to determine whether *Azotobacter* were present, 1 gm. of soil was inoculated into 100 cc. of sterile Ashby's medium, and incubated at 30°C. for 14 days. At the end of this period, the flasks containing the medium were examined and the nitrogen was determined by the modified Kjeldahl method. These results appear in table 1.

Since a large number of soils did not develop colonies under any treatment, certain changes were made in the method. Sackett and Stewart (17) recommend that sandy soils be treated with kaolin, that heavy soils be treated with sand, and that basic soils be treated with phosphoric acid in order to promote more favorable conditions. Accordingly a series of soils were treated as follows:

- A. Check—50 gm. soil + 2.5 gm. starch
- B. 50 gm. soil + 2.5 gm. starch + 0.15 gm.  $\text{K}_2\text{SO}_4$
- C. 50 gm. soil + 2.5 gm. starch + 0.3 gm.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

TABLE 1  
*Development of azotobacter colonies on plaques*

SAMPLE NUM- BER*	LOCATION	NUMBER OF COLONIES			N FIXED PER GM. MANNITE	REMARKS
		Check	$\text{NaHPO}_4$	$\text{K}_2\text{HPO}_4$		
					mgm.	
50	Oak Creek Canyon near Jerome. Orchard	0	0	0	9 2	Peach orchard producing well. No $\text{P}_2\text{O}_5$ added
53	Little Chino Valley, Prescott	0	0	2	6 1	First year out of desert, planted to peas. Poor growth
51	Big Chino Valley, Prescott	0	12	20	10.7	Plowed from desert, 1929. In corn, excellent growth and yield
43	Rigo Ranch, Prescott	0	0	0	8 2	Dry farmed 17 years, in corn continuously, good yields
26	Mojave Loam, Phoenix	19	100	60	9 0	Fallow
47	Edwards Farm, Clemancau	0	0	150	12.2	Garden. Heavily manured
31	Michelbach Ranch, Flag- staff	0	0	0	5 0	Potatoes. Poor growth and yield
30	Curry Ranch, Flagstaff	0	0	0	4 2	Heavy stand of oats
35	Duke Ranch, near Meteor Crater	14	30	9	11 8	Good stand of beans
36	Duke Ranch	30	8	20	6 5	Poor stand of beans
32	E. Burrus Ranch, Flagstaff	0	0	0	11 0	Potatoes—good growth
42	Coconino Nat. Forest, Flagstaff	0	0	0	5 7	Typical pine forest soil
46	Bochot vegetable garden, Prescott	0	0	0	9 5	Heavily manured. Excellent yield of vegetables
21	Glendale very fine sandy loam, Phoenix	50	160	80	11 4	Heavy stand of Sudan grass
24	Glendale sandy loam, Phoenix	100	300	400	11 0	Fallow
25	Mojave sandy loam, Phoenix	0	0	0	9 3	Fallow
28	Pima clay, Phoenix	50	400	400	11 5	Cotton. Fine growth
20B	Bradley Ranch, Gila Val- ley, Yuma	4	200	100	10 8	Heavy soil. Fair stand of alfalfa, due to salt accumu- lation
38	Edge of Painted Desert, near Winslow	0	0	0	1 0	Typical Red Mesa soil, scant growth, creosote bush, mes- quite
16	University Farm, Yuma	0	0	0	6 4	Sandy mesa soil, citrus. Good condition
13	Diller Ranch, Casa Grande	40	120	70	9 3	Very tight, impervious soil
27	Mojave Clay Loam, Phoenix	0	500	500	11 2	Heavy stand of wheat

\* Roman numerals designate soils used by McGeorge and Breazeale (11, 12) in some phosphate availability studies.

TABLE 1—*Concluded*

SAMPLE NUM- BER*	LOCATION	NUMBER OF COLONIES			N FIXED PER GM. MANNITE	REMARKS
		Check	Na <sub>2</sub> HPO <sub>4</sub>	K <sub>2</sub> HPO <sub>4</sub>		
17	Old Fertilizer Experiment plot, Yuma	200	60	120	9.4	Super phosphate plot citrus trees. Fine condition
37	Pessarra Ranch, Flagstaff	4	6	6	5 7	Good stand of beans
48	Verde Valley, near Jerome	0	0	0	11 5	Fair alfalfa in area damaged by smelter smoke
44	Goff Ranch, Dewey	5	15	15	9 1	Heavy stand corn
I	Tal-wi-wi, Phoenix	30	0	0	9 5	Grapes, heavy phosphate appli- cation
II	Same	0	0	0	10.2	No phosphate added
III	Bard, California	0	0	0	8 5	From plot deficient in phos- phate
IV	Davis Ranch, Tempe	0	25	100	10 7	Poor cotton, low in phosphate
V	Same	0	200	0	10.3	High in soluble P <sub>2</sub> O <sub>5</sub> does not respond to phosphate fertili- zation
VI	University Farm, Tucson	0	0	0	11 4	Black alkali soil. High in water soluble P <sub>2</sub> O <sub>5</sub>
VII	Turley Ranch, Tempe	0	150	150	11 0	Poor cotton. Low in soluble phosphate

D. 50 gm. soil + 2.5 gm. starch + 0.15 gm. K<sub>2</sub>HPO<sub>4</sub>

E. 50 gm. soil + 2.5 gm. starch + 0.085 gm. H<sub>3</sub>PO<sub>4</sub>

F. 50 gm. soil + 2.5 gm. starch + 10 gm. fine sand + 0.3 gm. Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O

G. 50 gm. soil + 2.5 gm. starch + 2 gm. CaSO<sub>4</sub> + 3 gm. Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O

Sackett and Stewart (17) state that soils of the "adobe type" must be aerated in order to produce good growth. Gypsum was added to determine whether it would coagulate soil colloids sufficiently to permit development colonies; a method used in the reclamation of "black alkali" soils. A soil of a pH 8.0 or greater would become "puddled" upon the addition of water, and this deflocculated condition would favor the development of anerobic nitrogen-fixing organisms at the expense of *Azotobacter*. The results of these various treatments are given in table 2.

#### DISCUSSION

Table 1 shows that out of 33 soils examined, 14 or approximately 42 per cent did not develop colonies under any treatment. Thirteen soils (or 39 per cent) showed a response to sodium phosphate applications, and 16 (38 per cent) responded to treatment with potassium phosphate. Only 7 soils responded more to potassium phosphate than they did to sodium phosphate. All soils, however, with the exception of No. 38 showed the presence of *Azotobacter*



when inoculated into Ashby's medium. This was demonstrated by the amounts of nitrogen fixed, and by characteristic membrane growth on the medium.

The few soils that did respond to additions of potassium and phosphorus, which indicates a deficiency of these elements, do not always correlate with crop growth. For example, No. 35 and 36 came from the Duke Ranch near Meteor Crater. The tract was planted in beans, yet the sample from the area of best growth gave more response to phosphorus than did the sample from an area of poor growth. Sample 51, which had been plowed from desert only 1 year before, and which had an excellent stand of corn which later gave a fine yield, responded to both phosphorus and potassium. Sample 47 responded only to  $K_2HPO_4$  yet this was a garden soil which had been heavily manured, and which gave good crop yields. Sample I and II gave no response, although I had been given heavy applications of phosphate which showed a crop response in the field, and II is the same soil, untreated. Samples IV and V reflect a similar

TABLE 2  
*Effect of soil treatments upon colony development*

TREATMENT	NUMBER OF COLONIES UNDER SOIL TREATMENT			
	VII	IV	V	VI
A	0	0	0	0
B	0	0	0	0
C	0	0	0	0
D	0	0	0	0
E	0	0	0	0
F	10	20	0	0
G	10	0	0	0

condition; IV, which was deficient in phosphate, did not respond so well to phosphate addition as did V, which contains an abundance of phosphorus, and which (in pot experiments) does not respond to phosphate fertilization. The table shows that only 17 of the soils (51 per cent) developed *Azotobacter* colonies under any treatment.

Table 2 shows that there was little response to various treatments, although samples VII and IV did respond to additions of sand. On the other hand, No. VI is a typical "black alkali" soil which contains relatively large amounts of soluble phosphate, yet it did not respond to any treatment, although table 1 shows that it contains an active *Azotobacter* flora.

It is apparent, then, that the proposed methods of Sackett are not applicable to Arizona conditions. In addition to the treatment used in table 2, other plaques were made using different carbohydrates as a source of energy, and by adding varying amounts of phosphorus in the forms of different salts. The results, however, were not encouraging.

It is probable that the lack of development of colonies when Arizona soils are used is due to a lack of aeration. It is very difficult to bring many of these alkaline soils to their optimum water content without a "puddled" condition resulting. Although Sackett recommends that  $H_3PO_4$  be added to basic soils (pH 8.4 or greater), the author does not recommend this procedure for Arizona soils. Because of their calcareous nature, the phosphoric acid is rapidly neutralized without bringing any significant change in pH. Furthermore, Burgess<sup>2</sup> has found that there is considerable nitrogen fixation in soils of high pH, as table 3 shows. In these experiments, 2 gm. of soil was inoculated into Ashby's medium, and incubated for 2 weeks at 28°C.; in another series 100 gm. of soil was placed in a tumbler, 1 gm. of mannite added, and incubated for 4 weeks at optimum moisture content. The anerobic fixation was determined by inoculating 1 gm. of soil into 100 cc. of Winogradsky's solution containing 1 per cent of glucose. These solutions were freed from oxygen by placing them in a Novy jar, evacuating, and then filling the jar with nitrogen gas. These results,

TABLE 3  
*Nitrogen fixation in soils of high pH (data of Burgess)*

SOIL	pH	N FIXED PER GRAM OF MANNITE		
		Ashby's solution	Tumbler	Anerobic fixation
		mgm.	mgm.	mgm.
13	9.55	9.3	10 0	2 1
17	9 0	9.4	21.0	4 3
1a	9.3	17 0	24.0	5 3
6a	9 0	14 9	12.5	4 2
11B	10 0	10 8	20 0	2.2
6C	9 5	14 3	16 5	4 2

which appear in table 3, show that at pH values as great as 9.5 or 10.0, there is active nitrogen fixation.

The work of McGeorge and Breazeale in this laboratory is constantly changing our ideas concerning the availability of phosphorus in calcareous soils. One of the most striking observations is that some of the "black alkali" soils contain more soluble phosphorus than do fertile soils from non-calcareous regions of the United States. Another observation is that aeration has a pronounced effect on phosphate availability. Consequently, the mixing of a heavy soil with sand, or a light soil with kaolin, as Sackett recommends, is sufficient alone completely to alter the availability of phosphorus in the soil.

In general, the results given in tables 1 and 2 do not agree with the description of crop growth. All of these samples were taken by the author, so the remarks concerning the crops are the result of personal observation. The phosphate content of soils I-VII has been determined by McGeorge by use of

<sup>2</sup> Unpublished data from this laboratory.

the electrodialysis method and by pot experiments. Of the 33 soils examined, only 4 (12 per cent) gave results which agree with field observations, and consequently might be an index of fertility needs.

Walker (19) obtained encouraging results with this method in Iowa; Sackett and others have employed it in Colorado; Niklas and his coworkers in Germany have recommended it; and Guittonneau (9) in France has claimed excellent correlation with field conditions. The results of these experiments show, however, that it has but slight value for determining the mineral deficiencies of Arizona soils. These aforementioned investigators were working with soils which were not as highly calcareous, so their positive findings are not applicable to the soils of Arizona, which are usually highly calcareous and often have high pH values and high salt concentrations. This is especially true in the light of the results of McGeorge and Breazeale, which are to be published later.

Pittman and Burnham (14) have not found this test to be reliable in Utah. They found only about 70 to 80 per cent agreement between the test and the field response. This was only in extreme cases where the soil was either very deficient or well supplied with phosphorus. In the intermediate ranges, the correlation was lower.

In spite of the results of various workers, the author is inclined to agree with Pittman and Burnham (14) that this is not a good test for potassium needs, and furthermore believes that it has never been definitely established that *Azotobacter* have the same food requirements as higher plants, or in the event that they do, certain requirements are so small that such a test can only be a rough index at best.

Greaves (7) states:

Potassium is essential to the higher plants and cannot be replaced entirely by related substances, yet Gerlach and Vogel early reached the conclusion that potassium and magnesium are not essential to the *Azotobacter* . . . . If these elements are essential, it must be in extremely minute amounts, for Vogel, using the purest chemicals obtainable, was able to prepare potassium-free media in which the *Azotobacter* developed. He did find, however, that potassium favors their development.

In order to determine the need of potassium by *Azotobacter*, the following experiment was conducted:

Flasks containing 100 cc. of sterile Ashby's medium were inoculated with suspensions of *Azotobacter*, incubated for 2 weeks at 30 C., and then analyzed for nitrogen. Another medium was prepared which had the same composition as Ashby's medium except that  $\text{Na}_2\text{HPO}_4$  was used in place of  $\text{K}_2\text{HPO}_4$ . The pH of each media was 7.8. This series was incubated under the same temperature as the cultures in Ashby's medium. The medium was prepared from C. P. chemicals and was tested for the presence of potassium.

Only traces (less than 0.5 p.p.m. K) were found. The results are given in table 4.

The quantities of nitrogen fixed are practically as great in the presence of minute traces of potassium as in Ashby's solution. This shows that the

amount of potassium required by the *Azotobacter* is extremely small. Assuming the weight of the surface  $6\frac{3}{4}$  inches of an acre of soil to be 2,000,000 pounds, 0.5 p.p.m. K would be only 1 pound of K an acre.

TABLE 4  
*Milligrams of N fixed by azotobacter per gram of mannite*

CULTURE	AMOUNT OF K PER GRAM OF MANNITE	
	Less than 0.05 mgm.	22 mg. (Ashby)
<i>A. agilis</i> . . . . .	12 6	13.3
<i>A. vinelandii</i> . . . . .	9.0	9.8
<i>A. chroococcum</i> . . . . .	9.6	9.6
<i>A. Beijerinckii</i> . . . . .	9.2	9 0
<i>A. vitreus</i> . . . . .	1.5	1.5
<i>A. Woodstownii</i> . . . . .	1.2	1 2

TABLE 5  
*Influence of phosphate on nitrogen fixation*  
(Data of Ranganathan and Norris)

CULTURE	MGM. $P_2O_5$	MGM. N FIXED PER GRAM OF DEXTROSE
<i>A. chroococcum</i>	0	0
	4 2	4 8
	10.2	5 0
	20.8	5.4
	41.6	5 6
	52.0	6 0
	104.0	6 0
50 (from an Arizona soil)*	0	0
	2	6.8
	4	8.0
	6	7.0
	8	5.5
	10	5.6
	20	5.0
	40	4.4
	80	6 0
	100	4 9

\* Data of Burgess.

Table 5, which is compiled from data of Ranganathan and Norris (15) and from the unpublished results of Burgess, shows the relation between increasing amounts of phosphorus and nitrogen fixation, by pure cultures of *Azotobacter*, in dextrose—Ashby's solution.

Although Ranganathan and Norris (15) did obtain increasing nitrogen fixation with increasing amounts of phosphorus, Burgess did not. The latter did show, however, that a small amount of phosphorus is necessary, as shown by the greatest fixation when 4 mgm. of  $P_2O_5$  was added. Although Burgess did not obtain increased fixation with additions of more than 4 mgm. of  $P_2O_5$  this should not imply any contradiction with the results of Ranganathan and Norris, but seems to point clearly to an adaptation of the *Azotobacter* in Arizona soils. They have become adapted to a minimum of available phosphorus, and hence respond to minute amounts. The author has shown that the *Azotobacter* flora of Arizona soils have adapted themselves to higher temperatures and can fix nitrogen at greater temperatures than can cultures from more temperate regions (8), so it does not appear too far fetched to assume adaptation to minute amounts of phosphorus.

Apparently the *Azotobacter* are less sensitive to phosphorus than to potassium. If the lower limit of *Azotobacter* growth is less than 2 mgm. of  $P_2O_5$  in 100 cc. of a 1 per cent dextrose-Ashby solution; at the same rate, the minimum quantity of available  $P_2O_5$  in the surface  $6\frac{2}{3}$  inches of an acre of soil would be less than 40 pounds, which is probably low enough to require phosphate additions in order to produce good plant growth.

It seems, then, that the value of the plaque method is limited chiefly to soils which are extremely deficient in phosphorus, which is in agreement with the findings of Pittman and Burnham (14).

#### SUMMARY

1. The *Azotobacter* (plaque) method of determining mineral deficiencies in soils has been applied to a number of soils of known crop producing powers which represent the principal agricultural sections of Arizona.

Thirty-three soils were examined; thirty-two developed characteristic *Azotobacter* films on Ashby's solution, and fixed considerable amounts of nitrogen.

Fourteen soils (42 per cent) did not develop *Azotobacter* colonies on the plaques under any conditions. Nineteen soils (57 per cent) developed colonies.

The mineral deficiencies of the soil as determined by the *Azotobacter* method definitely agreed with field observations only in the case of four soils (12 per cent).

The minimum potassium requirements of *Azotobacter* are so small that this test is not a good index of potassium needs of a soil.

*Azotobacter* appears to have a higher minimum requirement for phosphorus than for potassium. From the data submitted, this test is of value only in the case of soils extremely deficient in phosphorus.

This test is of little value in determining the potassium and phosphorus requirements of the soils of Arizona, which are characterized by relatively high pH, their calcareous nature, and an active *Azotobacter* flora which has become adapted to these conditions.

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# THE MICROBIOLOGICAL POPULATION OF PEAT<sup>1</sup>

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The problem of the occurrence and abundance of different types of microorganisms in peat has recently received renewed attention, primarily in connection with the rôle of these microorganisms in the formation of peat from the plants and plant residues inhabiting the peat bog or brought into it. This renewed interest is primarily due, first to the various theories concerning the origin of coal, which takes place presumably through the peat stage, especially whether the lignins or the celluloses of the plant residues form the mother substances of coal; and, second, to the renewed interest in the age-old problem, whether the decomposition of organic residues is microbiological in nature or chemical. It has usually been assumed in the past that peat is a sterile medium, and when it was demonstrated that numerous microorganisms are found in peat, the question was asked whether they are just accidental invaders or whether they play an important function in peat formation. This discussion is similar to others in the past whether certain processes are microbiological or chemical in nature. Since the time of Pasteur, when the famous battle was fought between the bacteriologist and the chemist concerning the nature of fermentation, discussions of a similar nature continued to come up whenever the rôle of microorganisms in a process previously little understood had to be established. The problem of the origin of peat and the rôle of microorganisms in its formation and transformation will occupy a certain definite place, even if only a modest one, in the history of microbiology.

The problem of the relation of microorganisms to peat formation can be definitely summarized by the following questions: 1. Are microorganisms found in different layers of peat bogs laid down many years ago? 2. If they are found there, how abundant are they, and are they in an active state? 3. Do they continue to decompose the peat? 4. What part have they played in the formation of peat from the plant residues? 5. What part do they still play in the processes of continuous transformation of peat, if such take place?

Waksman and Stevens (13) have already shown that there is no justification

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whatsoever for considering a peat bog as a sterile medium, as was frequently done by many botanists, geologists, and chemists, but that it is teeming with life. In certain bogs, as in the highmoor peats, there is not only no diminution in the number of bacteria with an increase in the depth of the peat formation, but frequently there may be a very decided increase. The bacteria found in the lower depths of the bog seemed to comprise largely certain specific types capable of growing in rather acid (of pH 3.8–4.0) media, and capable of living both aerobically and anaerobically. Evidence was submitted to prove that the processes of initial decomposition of the plants at the surface of the bog and their further transformation into peat are primarily microbiological in nature.

That study was undertaken without any preconceived idea of proving or disproving any particular theory. However, the results were used by Fischer (5) and Lieske (7), on the one hand, and the opponents of the "lignin theory of the origin of coal" (2, 9), on the other, to prove or disprove the lignin theory. These results and other studies carried out in this laboratory and dealing with the chemistry of peat (11, 12) tended to demonstrate that this theory is to a certain extent justified, but that it is very one-sided and that it tells but a part of a very complicated story. Although it is true that the lignin is very resistant to decomposition by microorganisms, especially under the conditions prevailing in the peat bogs, namely, under anaerobic conditions, it never makes up more than a part of the total organic constituents of the peat, even in the most advanced stages of decomposition. Further, although in certain peats, as in sedimentary and lowmoor peats, the cellulose tends to disappear as a result of its slow but gradual decomposition by microorganisms, a fairly large quantity of hemicelluloses is still left.

The amount of protein that may be present in certain peats is very considerable, making up frequently over 20 per cent of the total organic constituents of these peats. The parallel increase that may be observed between the protein content and the decrease in the concentration of cellulose is a direct result of microbial synthesis, which always accompanies microbial decomposition and energy utilization.

It should be emphasized here that the nature of the plants which give rise to peat and the conditions in the bog (such as reaction and mineral content) under which the decomposition of the plant residues takes place, are of the greatest importance in determining the nature of the peat produced. One cannot compare readily a lowmoor peat with a highmoor peat, since they differ botanically, chemically, and microbiologically. The lowmoor peats, as well as the forest peats, show very marked signs of microbiological decomposition, which can be characterized as follows: a very rapid reduction in the cellulose content to almost complete disappearance, depending upon the age of the peat layer; a marked reduction of the hemicelluloses, with the non-pentosan hemicelluloses (largely uronic acid compounds) predominating in the decomposed peat, as compared with their relative abundance in the initial plants; an in-

crease in the protein and ash content, as compared with the original plant residues, this increase being parallel with the decrease in the cellulose, a large part of the protein being a result of microbial synthesis; a marked increase in the content of lignin and lignin-like complexes. These peats offer a favorable medium for microbial development and, when drained, offer an excellent medium for the development of higher plants. The chemical composition of this type of peat tells the story of its formation, as well as the rôle of microorganisms in the processes involved.

Highmoor peats, which have been formed predominantly of sphagnum plants, on the other hand, show totally distinct chemical characteristics: they are rich in cellulose and hemicelluloses; whether as a result of the physical nature of the plants, or the specific nature of their chemical constituents, they are highly resistant to decomposition, especially the carbohydrates and the lignins. However, the organic nitrogenous compounds in the sphagnum plants can undergo rapid decomposition, with the result that a marked reduction in the protein content is found; because of the resistance to decomposition of the carbohydrates in the sphagnum plants, especially under anaerobic conditions, there is also only a very limited increase in the ash content, frequently preceded by a decrease. Only when a dry period prevails for a considerable period of time will the aerobic conditions and the introduction of a new type of vegetation result in a change in these properties, such as an increase in the ash and protein content, as in the case of the Grenhorizont; the lignin content gradually increases with the increase in age of the peat, and the cellulose content decreases. This type of peat, once it is fully saturated with water, is a very poor medium for bacterial development, although far from being sterile; it offers also a poor medium for the growth of higher plants, even when drained, unless the reaction is corrected by the use of lime, and the needed nitrogen and mineral constituents are added.

In order to illustrate the fact that the microbiological activities in a lowmoor peat are markedly influenced, if not completely controlled, by environmental conditions and by the presence of available energy, the results presented in tables 1 and 2 will suffice. In one experiment 500-gm. portions of fresh lowmoor peat, obtained from Florida, were partially dried down, so as to adjust the peat to different moisture content, and the microbiological activities, as measured by the  $\text{CO}_2$  evolution, as well as the amount of mineralized nitrogen, were compared. Certain definite differences were obtained (table 1), showing that by changing a single factor, such as moisture content, marked differences will be obtained in the rate of peat decomposition.

In another experiment, 100-gm. portions of lowmoor peat from Florida, containing 80 per cent moisture, were placed in flasks. Four flasks were left untreated, four were treated for 48 hours with toluol, four for 48 hours with ether, and four with 2 per cent HCl; the last set was then washed with water and brought back to original moisture. All the flasks were inoculated with fresh soil suspension and incubated for 20 days. The  $\text{CO}_2$  given off was measured

at frequent intervals. At the end of the incubation period, the  $\text{NH}_3$  and  $\text{NO}_3$  produced and the numbers of bacteria and fungi present in the different flasks were determined (table 2).

The results show clearly that the various modifications of the peat have a marked effect in changing it as a medium for microbial activities. The changes produced depend entirely upon the nature of the treatment. When the treatment brings about a suppression of the fungi, as in the case of the toluol-treated peat, there is a small increase in bacterial development accompanied by a small increase in  $\text{CO}_2$  evolution and nitrogen liberation. Where the treatment results in a condition favoring fungus development, as in the ether-

TABLE 1  
*Influence of moisture content upon the decomposition of peat\**

MOISTURE IN PEAT	$\text{CO}_2$ GIVEN OFF	NITROGEN MINERALIZED
<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>
81 5	820	6
80 2	1,183	10
71 3	2,966	82
52 8	2,886	92
33 3	775	26

\* 500 gm. of peat of 81.5 per cent moisture; incubation 188 days.

TABLE 2  
*Influence of treatment of peat upon its decomposition*

TREATMENT	$\text{CO}_2$ GIVEN OFF	$\text{NH}_3$ -N	$\text{NO}_3$ -N	TOTAL NITROGEN MINERAL- IZED	BACTERIA*	FUNGI*
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>		
Untreated. . . . .	36 1	2 34	11 18	13 52	21,000,000	235,000
Toluene-treated. . . . .	62 1	7 70	7 50	15 20	24,250,000	52,000
Ether-treated. . . . .	204 8	7 74	0 43	8 20	97,000,000	7,100,000
HCl-treated. . . . .	145 8	14.57	0 55	15 12	105,500,000	359,000

\* In 1 gm. of moist peat.

treated peat, there is a much greater increase in the rate of decomposition, as shown by the  $\text{CO}_2$  evolution, but this is accompanied by a reduction in available nitrogen, due to the extensive synthesis of fungous mycelium. The mild acid treatment results in a favorable effect upon the decomposition of the peat and in a marked bacterial multiplication. However, this is accompanied only by a small increase in the available nitrogen, due possibly to the fact that this treatment was also partly favorable to fungous development.

Without going into a discussion of the underlying chemical and microbiological phenomena resulting from these treatments, it is sufficient to emphasize the fact that the microbial development in peat is markedly influenced by the

physical and chemical conditions of the peat and by the environmental conditions.

As to the influence of the chemical composition of the highmoor peat upon its decomposition, the following experiment will be of interest (table 3). Fresh sphagnum plants were dried and divided into three parts: one part was left untreated; one part was treated with 4 per cent sodium hydroxide solution for 5 hours in flowing steam, to remove the lignin and some of the hemicelluloses; a third part was treated with the hot alkali solution followed by hydrolysis with 2 per cent hot hydrochloric acid, for 5 hours in flowing steam, to remove most of the remaining hemicelluloses. The preparations were washed with distilled water and dried. Five gram quantities of the dry sphagnum preparations were added to 150-gm. portions of sand placed in Erlenmeyer flasks.

TABLE 3

*Decomposition of sphagnum plants, as influenced by removal of certain chemical complexes\**

	CO <sub>2</sub> LIBERATED	CONTROL SUBTRACTED	AMMONIA FORMED	NO <sub>3</sub> -N LEFT	TOTAL MINERALIZED N PRESENT	NITROGEN LIBERATED (+) OR CONSUMED (-)	RATIO BETWEEN CARBON LIBERATED AS CO <sub>2</sub> AND NITROGEN CONSUMED OR MINERALIZED (C/N)
	mgm. C	mgm. C	mgm. N	mgm. N	mgm.	mgm.	
Control. . . . .	11 5		2 2	27 8	30 0	...	7 8
Sphagnum alone, without nutrients. . . . .	148 4	136 9	14 4	3 2	17 6	+17 6	10 1
Sphagnum + nutrients	142 9	131 4	15 4	27 6	43 0	+13 0	.
Sphagnum extracted with hot 4 per cent NaOH.	273 3	261 8	1 8	15 0	16 8	-13 2	20 7
Sphagnum extracted with 4 per cent NaOH and 2 per cent HCl	393 4	381 9	1 6	2 0	3 6	-26 4	14.5

\* These results are taken from experiments carried out by Mr. H. W. Reuszer of this laboratory.

Each flask, with the exception of the nutrient-free cultures, received 200 mgm. NaNO<sub>3</sub>, 100 mgm. K<sub>2</sub>HPO<sub>4</sub>, and 40 cc. of distilled water. All the flasks were inoculated with a suspension of good garden soil, placed in an incubator at 27-28°C. and connected with a respiration apparatus. At the end of 46 days' incubation, the flasks were discontinued and the ammonia and nitrate nitrogen determined. The results show that the decomposition of the untreated sphagnum plants proceeds very slowly, since only about 145 mgm. of carbon were given off as CO<sub>2</sub> from the decomposition of 5 gm. of material, in a period of 46 days. However, the fact that as a result of this decomposition, nitrogen was also liberated in appreciable quantities and the fact that the addition of available nitrogen as nitrate had no favorable effect upon the rate of decomposition shows: 1. that the nitrogenous complexes in the sphagnum plants decompose

very readily, 2. that the carbohydrates (cellulose and hemicelluloses) are resistant to decomposition, 3. that because of this, little microbial synthesis takes place, and 4. as a result of this, the liberation of nitrogen in an available form takes place. The sphagnum plants which have been treated with an alkali and especially the material treated with both the alkali and acid, whereby most of the lignin and hemicelluloses were removed (nearly 80 per cent of the material was removed by both treatments), decomposed much more readily, as shown by the rate of  $\text{CO}_2$  evolution and nitrogen consumption. In this respect the sphagnum material behaved as residues of higher plants low in nitrogen usually do.

Thaysen (9) considers the existence of bacteria in deep layers of peat, laid down thousands of years ago, as totally anomalous; such an attitude toward the ability of anaerobic bacteria to exist in an anaerobic medium for long periods of time is totally unjustified; this idea is on a par with the claim of "the presence in the water of the bog of substances such as organic acids . . .", although nobody established the presence or formation of such acids in any significant amounts. The non-existence of such acids has been shown by Baumann and Gully (1). It is not only the acid conditions of the highmoor peat that make it a poor medium for the growth of microorganisms but the resistance of the organic substances of the sphagnum and other plants to decomposition. The lowmoor peat, which is much less acid, and which has originated from plants more readily decomposable usually contains a much more abundant flora of microorganisms. However, even the acid peat harbors an extensive flora of bacteria capable of living under the acid conditions prevailing in the bog, as shown later.

The numbers of bacteria found in different depths of a lake peat from Florida are given in table 4; the surface of this peat was 90 cm. below the surface of the water. The results show quite definitely that peat contains appreciable numbers of bacteria capable of living under aerobic and anaerobic conditions. Although these organisms are most abundant in the surface layers of the peat, where the organic residues are of recent origin and, therefore, undergo active decomposition, still the lower layers contain appreciable numbers of bacteria. This peat was free from fungi even in the surface layers, since the latter were deep under water. However, in the case of lowmoor peats, especially those that are partly drained, the surface of the peat will be found to contain large numbers of fungi and especially actinomyces (table 5).

In arguing against the activities of the bacteria in the older layers of peat, Thaysen questions their functions, since only few cellulose-decomposing bacteria, if any, were found. In this respect one must always examine the chemical composition of the peat; one can hardly expect many cellulose-decomposing organisms in a medium free from cellulose. The methods used for determining the presence and abundance of anaerobic cellulose-decomposing bacteria in a certain natural substrate have been one of the stumbling blocks in bacteriology. As a matter of fact, even after 80 years of investigation, since

Mitscherlich first announced the discovery of the anaerobic cellulose-decomposing bacteria (which later proved to be incorrect), even 30 years after Omeliansky's classical studies on these bacteria, we are still uncertain as to the actual isolation of pure cultures of these organisms, notwithstanding the excellent work of Khouvive, Warner, Fred, and others. The methods for demonstrating the presence of cellulose-decomposing bacteria, especially of anaerobic forms, depend largely upon the natural medium in which these organisms grow, such as soil, animal digestive tract, sea water, stable manure, and peat bogs.

TABLE 4  
*Distribution of bacteria in the peat profile of Algal Lake, Florida*

DEPTH OF LAYER	MOISTURE CONTENT	pH	ASH IN DRY MATERIAL	AEROBIC BACTERIA		ANAEROBIC BACTERIA	
				1 gm. of moist peat	1 gm. of dry peat	1 gm. of moist peat	1 gm. of dry peat
<i>cm</i>	<i>per cent</i>		<i>per cent</i>				
Surface*	97.8	7.5	18.8	360,000	16,820,000	200,000	9,090,000
60	97.8	7.6	24.4	82,000	3,727,000	150,000	6,820,000
90-120	90.9	7.3	39.6	25,000	275,000	80,000	879,000
150-180	91.5	7.1	23.3	28,000	329,000	180,000	2,118,000
210-270	95.5	7.2	8.8	60,000	1,334,000	160,000	3,556,000
300-360	94.2	7.3	14.7	450,000	7,930,000	180,000	3,103,000

\* 3 feet below surface of water.

TABLE 5  
*Numbers of microorganisms in an undrained peat bog in Florida*  
Numbers in 1 gm. of moist peat

DEPTH OF PEAT	MOISTURE	AEROBIC BACTERIA	ACTINOMYCES	FUNGI	ANAEROBIC BACTERIA
<i>cm</i>	<i>per cent</i>				
2-20	80.1	890,000	370,000	20,000	120,000
22-5	82.9	960,000	290,000	10,000	180,000
45	85.3	410,000	100,000	7,000	180,000
75	84.0	18,000	13,000	330	16,000
120	85.4	30,000	330	0	75,000
165	87.4	235,000	3,330	0	380,000

One is fortunate enough in being able to demonstrate the presence of these organisms, even if in limited numbers. Positive results are definite proof that the organisms are present. Negative results do not always necessarily prove that the organisms are absent. One would be more inclined to think that negative results may possibly be due to the inappropriate methods employed in searching for these organisms.

In the case of two typical lowmoor peats, one obtained from Newton, New Jersey, and the other from the Everglades, Florida, cellulose-decomposing bacteria have been demonstrated throughout the profile, including the lowest lay-

ers. The fact that these organisms decrease in numbers with depth can probably be accounted for by the mere traces of cellulose left below the surface of these peat formations. The fact that these organisms are found in the lower layers of these peats may be due not only to the traces of cellulose present, but to the ability of some of them to attack hemicelluloses as well, as was found to be the case of various cellulose-decomposing bacteria in the soil (6).

Another observation, which may prove to be of considerable importance for our knowledge of the decomposition of the organic complexes in lowmoor and sedimentary peats, is the discovery of certain actinomycetes, which have been isolated from depths of 135 cm. below the surface of the bog in the Everglades, Florida, or about 100 cm. below the surface of the water table. These actinomycetes are facultative anaerobic, have an optimum temperature at 40–42°C., attack cellulose, and are capable of attacking the organic complexes of the peat, under anaerobic conditions, without liberating any gases. Plate 1 illustrates the abundance of actinomycetes mycelium that may be found in peat. This particular clump of peat came from a surface layer, however. The determination of the abundance of actinomycetes by the plate method gives only a very faint idea of the extent of development of these organisms.

These results may be considered as definitely establishing the occurrence of cellulose-decomposing microorganisms throughout the profile of the lowmoor peat bogs as well as the fact that the removal of the cellulose, in the process of formation of these peats, is due entirely to the action of microorganisms.

Sedimentary peats and forest peats present somewhat different relationships in that the former contain very little cellulose even at the beginning of the process of peat formation, whereas the latter contain some cellulose even in the peat stage, which is still in the process of active decomposition.

The most interesting problem, however, in connection with peat formation and peat decomposition, is that of the highmoor peats. This is due both to the abundance of these peats in the northern regions of Europe and to the fact that they have been largely used by those investigators who usually reported negative results concerning the activities of microorganisms in peat formation. A highmoor peat consists of a layer of sphagnum peat, of varying degrees of thickness, formed directly upon an inorganic substrate, or superimposed upon a layer of sedimentary peat (Gyttja, Dy), sedge or reed peat, or forest peat.

An analysis of the chemical composition of peat in different depths of a highmoor profile (12) will reveal the fact that chemically the nature of the particular layer of peat depends entirely upon the plants and plant residues from which it has been formed. The sedimentary layer, the sedge or reed peat layer, and the forest peat layer in highmoor peats will be similar in composition to that of the corresponding peats, namely the sedimentary, lowmoor, and forest peats, as shown by the low cellulose content, high mineral content, high protein content, and high lignin content. On the other hand, the sphagnum layers, usually many feet thick, which give the characteristic properties to the

highmoor peats, are distinctly different in chemical composition. The sphagnum layers are rich in cellulose and hemicelluloses; they are low in ash and in protein; they contain a moderate concentration of lignin or lignin-like substances, depending upon age; they are distinctly acid in reaction. As a matter of fact, on comparing the chemical composition of sphagnum peat with fresh sphagnum plants, from which such peat originated, one will observe that, with two exceptions, the relative composition is quite similar, unless the particular layer of peat has undergone aerobic decomposition due to a continuous dry period, as in the formation of the Grenzhorizont. The two exceptions are the lower nitrogen content and the greater lignin content; the former is due to the presence of readily decomposable nitrogenous compounds in the sphagnum plants and the difficultly decomposable carbohydrates, as a result of which there is little synthesis of microbial cell substance.

These phenomena can readily explain the difference in the nature of the microbial populations in the various peats. The sphagnum layers of the highmoor peat, below the surface layers, are poor in microorganisms, especially in cellulose-decomposing bacteria. This is due largely to the fact that the reaction (pH 4.0 or less) is highly unfavorable to the growth of such bacteria (4). Further, the specific form in which the cellulose and hemicelluloses exist in the sphagnum plants renders them highly resistant to the action of such bacteria. The anaerobic conditions are unfavorable to the development of the fungi. However, one is not justified in applying these facts to peat bogs in general and even to all the layers of highmoor peats. In order to show that such generalizations are not only unjustified, but quite wrong, further experimental evidence will be presented. For this purpose, certain of the studies on the distribution of microorganisms in the highmoor peats from Maine, carried out in the summer of 1928, were repeated again in 1931. One of these bogs, namely the Cherryfield or Denbo heath, is known to have never been disturbed by man.

The Denbo heath (3), located northwest of Cherryfield, Maine, has a typical dome-shaped surface, which rises from the margin of the bog to the center. The vegetation of the bog is made up largely of various species of *Sphagnum* (*S. tenellum*, *S. acutifolium*, *S. megallanicum*), with an admixture of species of *Eriophorum*, *Scirpus*, *Vaccinium*, *Drosera*, etc. The bottom of the bog, where the samples were taken, was reached at about 21 feet. The sphagnum peat extended to a depth of 15 to 17 feet; below that there was a layer of sedge peat superimposed upon a sedimentary layer (detritus gyttja), with a compact sandy bottom.

The Orono or Veazie bog is located outside of Orono, Maine. It is only slightly convex, is a shallower bog, and is not a typical highmoor. The *Sphagnum-Eriophorum* layer is only about 5 to 6 feet thick, superimposed upon woody peat with a clay-gyttja bottom, upon compact sand, the whole bog being about 12 feet deep.

Samples were obtained from a number of depths of these two bogs and put into a series of sterile glass jars. For bacteriological studies the inner part of



the core was used. However, the fact that the numbers of bacteria do not diminish with depth, but markedly increase (table 7) is sufficient evidence that no contaminating material could have been brought down with the sampling tube from the surface.

The reaction, moisture, ash, and nitrogen content of the Denbo profile are given in table 6, and the distribution of microorganisms in this profile is shown

TABLE 6  
*Physical and chemical conditions of the Cherryfield or Denbo peat profile*

DEPTH OF PEAT	MOISTURE CONTENT	pH	ASH IN DRY PEAT	NITROGEN IN DRY PEAT
<i>cm.</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
Surface	82.1	4.0	4.24	0.875
5	89.5	4.1	1.52	0.595
30	91.7	4.0	1.07	0.527
45	90.9	4.0	1.53	0.777
75	92.4	3.9	0.92	0.754
195	94.3	4.2	1.56	0.620
330	94.4	4.2	1.83	0.586
450	93.8	4.3	1.61	0.644
570	85.9	5.0	88.47	0.624
570	70.9	5.2	74.55	0.555

TABLE 7  
*Chemical composition of the Denbo peat profile*  
On per cent basis of dry material

DEPTH OF LAYER	ETHER-SOLUBLE	HOT WATER-SOLUBLE ORGANIC MATTER	HEMICELLULOSES	CELLULOSE	LIGNIN
<i>cm.</i>					
Surface	2.43	2.15	22.60	16.04	30.85
5	2.70	1.61	24.34	17.09	25.20
30	1.93	2.06	25.94	16.06	24.71
45	2.30	1.52	22.36	14.02	29.30
75	2.47	1.16	21.41	14.83	28.73
195	.	.	.	.	.
330	5.07	2.09	17.46	12.33	34.44
450	5.04	2.60	14.97	10.87	37.04
570	0.65	0.22	Trace	Trace	8.35
570	1.55	0.52	1.83	Trace	17.67

in table 7. The reaction remains more or less uniform (pH 3.9-4.2) throughout the depth of the sphagnum layer; this is accompanied by a uniformly low ash and nitrogen content. However, as soon as the reaction of the peat increases above pH 4.3, or becomes less acid, there is an accompanying increase in the ash and nitrogen (on ash-free basis) content, showing that a change of the sphagnum peat into a sedge or reed peat, or into a forest or sedimentary peat, has taken place. This has been confirmed by botanical examination.

The chemical composition of this peat profile is given in detail in table 7. However, the important point to emphasize in connection with this profile is the change in the abundance of microorganisms with a change in the chemical composition of the peat (tables 7 and 8).

As long as the reaction of the bog is pH 4.0 and the peat is the typical sphagnum of the highmoor type, the numbers of bacteria remain low. However, as soon as the reaction becomes less acid and the vegetation from which the peat has been formed changes, there is a rapid rise in bacterial numbers. These numbers were determined by the plate method, using a medium consisting of 5 gm. peptone, 10 gm. glucose, 0.5 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 gm.  $\text{K}_2\text{HPO}_4$ , and agar. By the use of the shake tube method, large numbers of anaerobic, gas-forming and non-gas forming bacteria were also obtained. Plate 2 gives a pictorial representation of the relative distribution of bacteria with

TABLE 8  
*Distribution of bacteria in a highmoor peat profile in Maine (Cherryfield)*

DEPTH OF PEAT  cm.	NUMBERS OF MICROORGANISMS PER 1 GM OF MOIST MATERIAL		BACTERIA IN 1 GM. OF DRY MATERIAL
	Bacteria	Fungi	
Surface	50,300	57,000	281,000
5	197,000	30,000	1,876,000
30	33,000	2,000	400,000
45	21,600	1,000	237,000
75	766,000	0	10,350,000
195	2,260,000	0	39,650,000
330	3,293,000	0	58,800,000
450	3,006,000	0	48,500,000
570	5,513,000	0	39,100,000
570	4,800,000	0	16,500,000

increasing depth of the Denbo peat bog, using a dilution of 1:50,000 on the fresh moist peat. The actual numbers are reported in table 8.

During the process of taking the samples of peat, when the borer was introduced into the bog, and especially when a depth of about 350 cm. was reached, as well as below that layer, abundant gas evolution took place. This gas took fire readily. Although small quantities of gas came up also from the higher, or sphagnum layers of the peat, the most abundant evolution of the gas seemed to coincide with the abundant development of bacteria, as shown by the plate method, and with the presence of anaerobic gas-forming bacteria, as determined by the shake tube procedure.

The chemical composition of the Orono peat is given in table 9, and the numbers of bacteria are reported in table 10. A change from the sphagnum to the sedge and forest peat is found at a depth of between 60 and 180 cm. This is accompanied by a change in ash and nitrogen content, in the composition of the organic constituents, and in the numbers of microorganisms.

These results should entirely dispose of the unjustified and misleading assumption of the sterility of peat, as well as of the hypotheses concerning the purely chemical processes involved in the transformations that presumably predominate in peat bogs. The authors do not want to deny in the least that, under certain conditions, as in the sphagnum layers of the highmoor peats, chemical processes, especially those of weak hydrolysis and reduction, may take place, as a result of the great resistance of these organic complexes to microbial decomposition and of the unfavorable conditions for the abundant development of many organisms. There is no need, however, for assuming, as

TABLE 9  
*Physical and chemical conditions of the Orono peat profile*

DEPTH	MOISTURE	pH	ASH IN DRY MATTER	NITROGEN IN DRY MATTER
<i>cm.</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
3-5	96.9	4.0	6.34	0.665
20	92.3	4.2	2.04	1.057
60	95.7	4.6	2.04	0.938
180	94.4	5.3	5.71	1.604
300	92.0	5.8	12.31	1.765
330	79.8	6.1	68.42	0.690
330-360	72.5	6.0	70.72	0.425

TABLE 10  
*Distribution of bacteria in a highmoor peat profile in Maine (Orono)*

DEPTH OF PEAT	BACTERIA IN 1 GM. OF MOIST PEAT	BACTERIA IN 1 GM. OF DRY PEAT
<i>cm.</i>		
3-5	27,600	890,000
20	230,000	3,240,000
60	7,000,000	162,800,000
180	4,080,000	72,800,000
300	5,100,000	63,700,000
330	7,000,000	34,600,000
330-360	8,000,000	29,100,000

Lieske (8) has done, that in such bogs, the celluloses are hydrolyzed chemically to sugars and the latter enable the organisms to grow. That such an assumption is also totally unjustified is revealed by an examination of the data on the chemical and microbiological conditions of the lower layers of the bogs. But even in the sphagnum layers, this assumption is not justified, because of their very high cellulose content.

A number of cultures of bacteria, largely facultative anaerobic forms, characteristic of the various types which developed on the plate or in the tubes, were isolated, and grown in pure culture. Since these bacteria were isolated from samples of peat taken from various depths of the highmoor and lowmoor pro-

files, they are believed to represent typical bacteria inhabiting those peats. Most of these bacteria produce gas when grown in liquid media containing glucose. Although there is no doubt that some of these bacteria have never before been described, the mere naming of organisms, the functions of which in natural processes still remain to be determined, is of questionable value. The group of cultures were, therefore, studied only in a very general way, without any attempt to describe them in detail.

Most of them were non-spore-forming short rods, with rounded ends, to almost spherical organisms, about a micron in diameter and a little over a micron long. Most of these bacteria were gram-negative, non-motile or motile. Some were gram-positive. A few larger rods, about  $2\mu$  long, forming diploids, were also found. Physiologically they were characterized by the readiness with which they grew in acid media, of pH 4.0. Some were proteolytic, as determined by their ability to liquefy gelatin and digest casein in milk, others were only weakly proteolytic. Some produced active diastatic enzymes, others did not. Most of them grew readily on synthetic media with glucose or sucrose as sources of energy. When freshly isolated, some produced on synthetic media (glucose agar) and on nutrient agar, an abundant cream-colored to brown and frequently dark-brown, slimy growth. They were isolated from dilutions ranging from 1,000 to 10,000, from all depths of the peat profiles, especially from the sedge and reed layers, usually at a depth, in the case of the highmoor peats, of 165 to 540 cm.

In addition to the facultative anaerobic bacteria, certain yeasts and various obligate anaerobes were isolated from the peats. No attempt will be made to describe these organisms either. The anaerobes were found to be rod-shaped, spore-forming or non-spore-forming, motile or non-motile organisms. Several cellulose-decomposing bacteria were isolated. This could best be accomplished by spreading some peat in a sterile Petri dish and pressing upon the surface of the peat strips of sterile filter paper. If cellulose-decomposing bacteria are present, they will attack the paper, changing it to a slimy consistency and producing rose, pink, yellow, and dark pigments on the paper.

The common procedure of inoculating liquid or solid (especially silica-gel) media containing paper as the only source of energy with the various peat materials frequently gives negative results, which cannot be taken as definite proof of the absence of such organisms. The media are to be considered as unsuitable for the development of such organisms. By modifying the composition of the medium, positive results are frequently obtained where negative results were found by the use of synthetic media. The occurrence of such bacteria at lower depths of undisturbed peat bogs can only prove that these bacteria are active in the peat in bringing about a slow and gradual decomposition of the cellulose. Some of these bacteria are being studied in further detail.

## SUMMARY

1. A study has been made of the occurrence of bacteria in different layers of a number of lowmoor, sedimentary, and highmoor peat profiles.

2. The idea prevalent in some quarters that peat bogs are sterile below the surface has been shown to be entirely wrong. An abundant population consisting of bacteria and, in the case of certain lowmoor peats, of actinomyces was found at all depths of the peat profiles.

3. In the case of the highmoor peats, the sphagnum layers contain only a relatively limited bacterial population. However, below these layers, or as soon as the forest, sedge, or sedimentary layers of the peat are reached, there is a great increase in bacterial numbers.

4. The bacteria found in the lower depths of the highmoor peat profiles are autochthonous, or native to their medium, and have not been brought there by an outside agency. They find in the anaerobic system of those peats as natural a substrate as the aerobic bacteria find in soil or elsewhere, where free oxygen is admitted.

5. The fact that the greatest numbers of bacteria were found in those layers where the greatest decomposition has taken place indicates that these bacteria are largely concerned with the process of decomposition.

6. The existence of cellulose-decomposing bacteria has been established not only in lowmoor and forest peats, but also in highmoor peats. The fact that in the latter the reaction is not very favorable for the development of these organisms and the fact that the cellulose and hemicelluloses of the sphagnum plants are highly resistant to decomposition by microorganisms, account for the very slow disintegration of the sphagnum plants in the process of peat formation. This is further substantiated by the fact that, although sphagnum plants have only a relatively low nitrogen content (about 1 per cent of the dry weight), a part of this nitrogen will be liberated as ammonia when the decomposition of the sphagnum plants takes place, as a result of the inability of the microorganisms to attack readily the carbohydrates of these plants.

7. Results have been submitted which prove beyond doubt that not only are microorganisms present in peat laid down many centuries ago; not only are they found there in great abundance, but that their abundance is closely correlated with the decomposition of the peat-forming plants, as well as the gradual changes still taking place in the peat itself.

8. These results tend to prove that microorganisms, largely bacteria, are chiefly responsible for peat formation and peat transformation.

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## PLATE 1

GROWTH OF ACTINOMYCES IN LOWMOOR PEAT PARTIALLY DRAINED

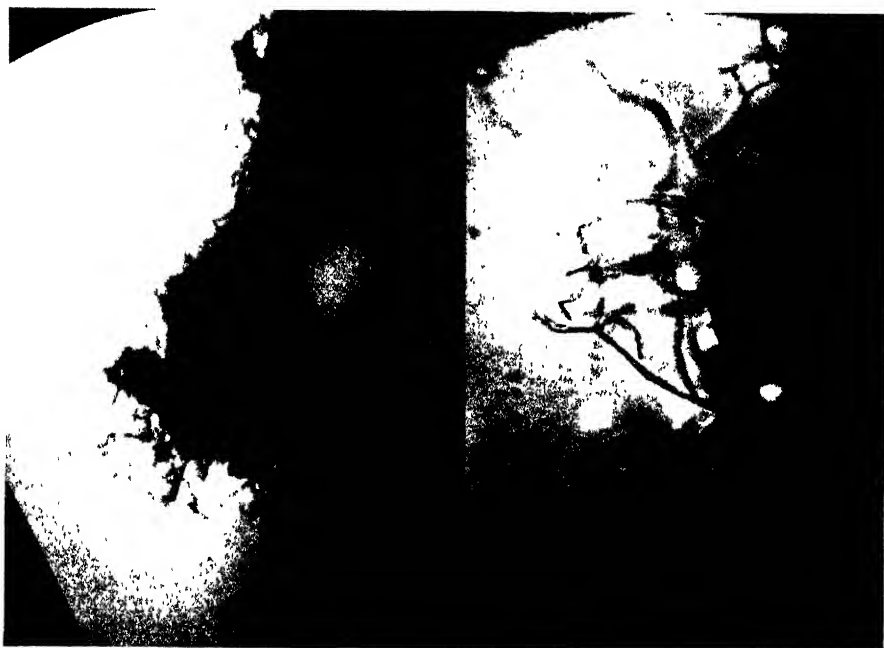


FIG. 1a

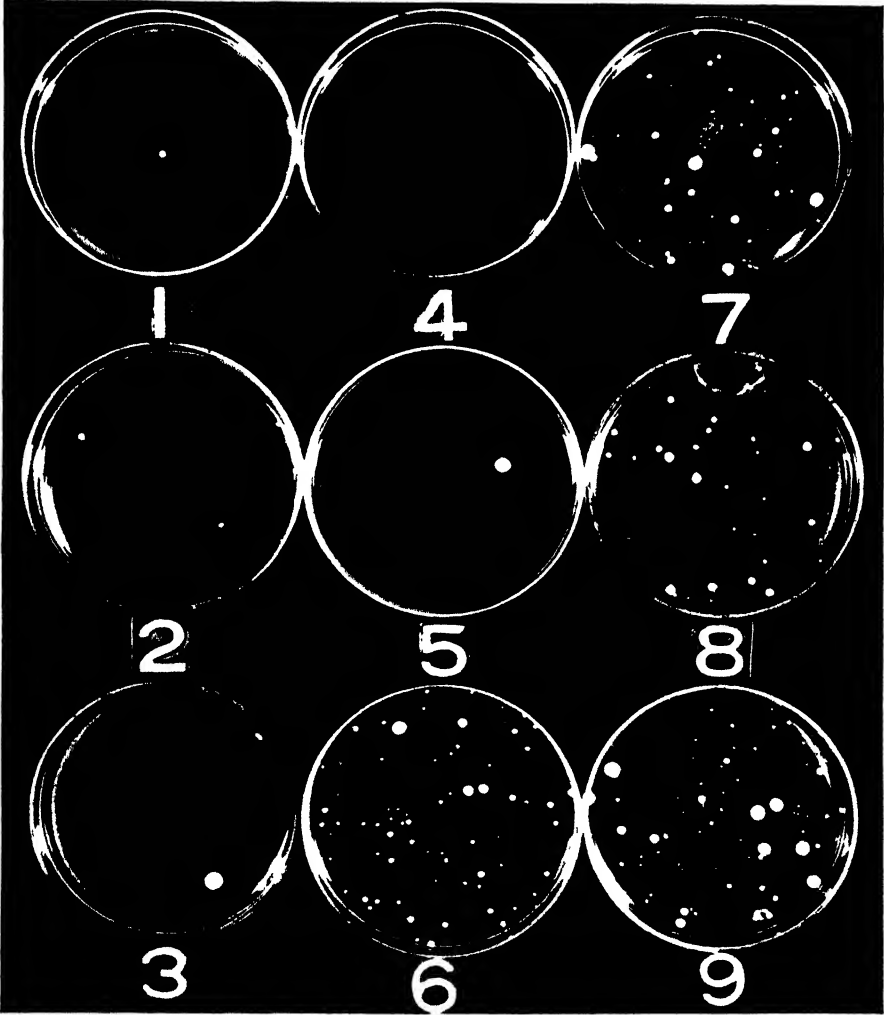
FIG. 1b



## PLATE 2

DEVELOPMENT ON PLATES OF BACTERIA FROM DIFFERENT DEPTHS OF A HIGHMOOR  
PEAT PROFILE

Dilution in all cases, 50 000, moisture content is given in table 6, depths 1-surface, 2 5 cm., 3-30 cm, 4-45 cm., 5-75 cm., 6-195 cm, 7-330 cm, 8 450 cm, 9-570 cm.





# A NEW MANOMETRIC APPARATUS FOR THE MECHANICAL ANALYSIS OF SOILS AND OTHER DISPERSE SYSTEMS

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The subject of mechanical analysis of soils has received a good deal of attention from soil workers during recent years. The modern tendency has been to express the mechanical composition of a soil in the form of a continuous curve, and many ingenious methods have been suggested for this purpose. For a description of the various methods of determining the size distribution of soil particles, the reader is referred to an excellent résumé of the subject by Odén (4), whose automatic balance (later known as the Odén-Keen balance in its improved form) is well known.

Of the more recent ones may be mentioned the hydrometer method of Bouyoucos, which consists in placing a specially made hydrometer in the soil suspension and taking readings at various intervals of time (1). The technique of the method is similar to that used by Pratolongo as far back as 1917 (5), who employed a Westfal balance instead of the hydrometer. The principle of the method was first enunciated by Wiegner (9), and depends on the variation of the density or hydrostatic pressure with the time at a definite distance from the surface in a sedimenting column.

Bouyoucos' method though attractively simple is subject to grave errors, which have been pointed out by Keen (3). These errors are avoided in the new type of hydrometer devised by the writer and described elsewhere (7). It consists of a short bulb (6-8 cm. long), and a long thin stem (50-60 cm. long), the readings being taken with a special device accurate to the fraction of a millimeter. Wiegner's original method of balancing liquids and its subsequent modifications suffer from many serious defects, the chief of which are the small variations in the height of the suspension and the measuring tube, and the sensitivity of the latter to small variations in temperature. These defects have been partly overcome by Odén by the use of a liquid of low specific gravity in the measuring tube and immersing the latter in the suspension. The experimental technique however still remains tedious.

Crowther has described an apparatus for the direct determination of summation percentage curves in mechanical analysis (2). It is based on the

<sup>1</sup> This work was carried out by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the council. Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

principle that the difference in hydrostatic pressure between two points separated vertically by a distance which is small compared with the total depth of the sedimenting column, may be taken without serious error as the density at a point midway between them. Although the variation in the hydrostatic pressure between such points during the sedimentation of a dilute suspension is small, it is possible to measure it by the help of a highly sensitive micromanometer. From the data given, however, it is doubtful whether the apparatus is capable of a high degree of sensitivity, for instance a 1 per cent suspension of sand is shown to give a maximum difference in the manometer of 1.2 cm., which is equal to a little more than 8 per cent of any fraction for 1 mm. difference in the manometer and as this represents actual difference in the levels of the manometric liquid, the movement of the meniscus would be only half of that. The magnification can be increased by using a suspension of higher concentration, but it is not entirely free from objection besides having its limitations. No data are given to show how far the method compares with, say, the pipette technique.

The following apparatus will be found useful for routine analysis as well as for work requiring greater precision.

#### DESCRIPTION OF THE APPARATUS

The apparatus shown diagrammatically in figure 1 is based on the principle of the differential liquid manometer, having a mixture of aniline and benzene (of density slightly higher than that of water) as the heavier liquid, and water itself as the lighter liquid. Two cylinders of equal diameter and fitted with ground glass stoppers, are used, one to hold the soil suspension and the other water, filled to the same height as the suspension. By the help of a three-way tap the connection between the suspension and water column can be established directly or through the manometer as desired. The lower end of the tube that dips into the suspension is spiral shaped so that if the suspension enters, it cannot rise above the level of the tip.

The suspension is well shaken and the cylinder placed alongside the one containing water. The apparatus is then lowered to the desired depth and direct connection made between the two cylinders. Since the suspension can support a higher column of water, the level in the water cylinder rises by a few millimeters depending upon the concentration of the suspension and the depth to which the end of the tube has been lowered. After about 3 minutes the tap is turned off and the apparatus taken out. If too much suspension has entered the spiral tube it is washed out by dipping the other end into a cylinder of water and syphoning it out. The aniline-benzene meniscus is also brought to the lowest point on the manometer tube adjoining the suspension cylinder. The suspension is again shaken and set aside just when a stop watch is started. Since a preliminary levelling of the liquids in the cylinders has already been effected, the connection is established through the manometer. At first there is a slight depression of the meniscus in the tube by the side of the suspension,

which soon begins to rise, and steadily moves up as the particles settle down and its level is noted at suitable intervals of time as explained subsequently either directly by a scale attached to the manometer tube or by the help of a kathetometer.

If  $D$  is the internal diameter of the cylinders and  $d$  of the manometer tube, the magnification is  $D^2/d^2$ . For a 1 per cent suspension which has a density of 1.0063, the level of the water will be  $0.0063v$  cm. higher than that of the

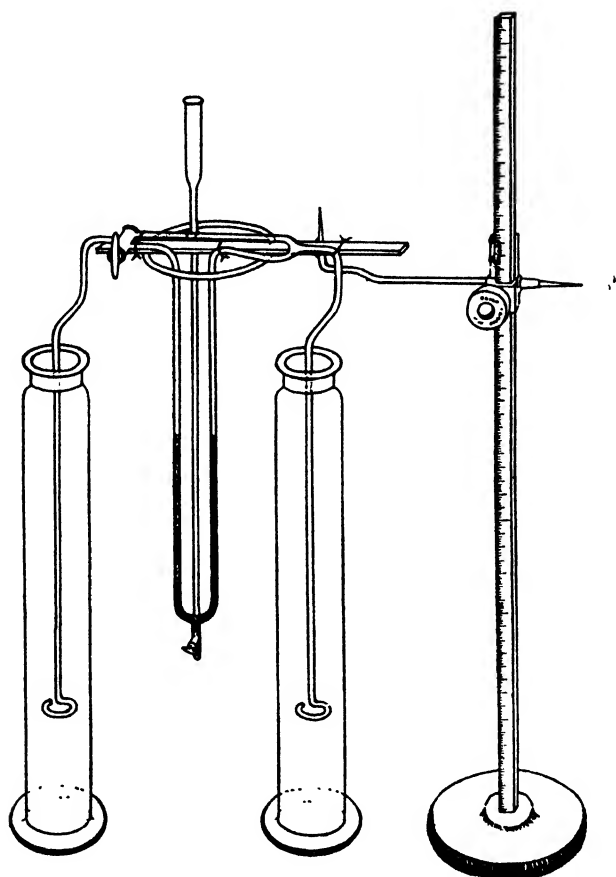


FIG. 1. DIAGRAM OF THE MANOMETRIC APPARATUS FOR THE MECHANICAL ANALYSIS OF SOILS

suspension when the side tube is dipping  $v$  cm. below the surface of the suspension. To produce this difference, however, when both the cylinders are connected, the fall in the suspension level and the corresponding rise in the water level must be equal to  $0.0063/2$  cm.; and, vice versa, the same rise and fall must take place in the opposite direction when the whole of the suspension settles down. However, this change in the levels of the cylinders will correspond to a movement of the meniscus in the manometer of  $D^2/d^2 \cdot 0.0063 v/2$

cm. or when 1 per cent of the total solids in the suspension falls down the manometer will register a change of  $D/d \times 0.0063 v/2 \times 100 = a$  cm. In other words the percentage of any fraction settled down between times  $t$  and  $t'$  from the beginning of sedimentation will be obtained by dividing the change in the meniscus reading during the above time interval by  $a$ . As long as the sedimentation cylinders used are of the same diameter,  $a$  remains constant. If a 2 per cent suspension is used then obviously the change in the manometer readings must be divided by  $2a$  and so on. Similarly if the depth of the sedimentation column is altered from  $v$  to  $v'$  the readings are to be divided by  $av'/v$ .

It will be seen that the magnification of the instrument depends upon the size of the cylinders, the concentration of the suspension, and the depth of the sedimenting column. By a judicious combination of these the desired magnification to suit any particular requirement might be obtained. In the writer's instrument the manometer registered a total change of 33.3 cm. for a 2 per cent suspension when the side tube was kept at 30 cm. depth, or a change of 3.3 mm. corresponded to the settling of 1 per cent of particles. The manometer tube had an internal diameter of about 6 mm. and the cylinders were about 10 cm. in diameter and about 45 cm. in height.

#### INTERPRETATION OF RESULTS

The method of calculating the results is the same as for Wiegner's apparatus, the mathematical treatment of which is given in Odén's paper (4). The following exposition may be found simpler.

Supposing a uniform suspension of soil consists of particles of equivalent diameter  $d_1, d_2, d_3, d_4, d_5 \dots$  and supposing the concentration of the various groups of particles is  $A, B, C, D, E \dots$  respectively and the time taken by each individual group to settle 10 cm. is  $t_1, t_2, t_3, t_4, t_5 \dots$

If the side tube is dipped in the suspension to a depth of 10 cm. and readings of the meniscus are taken after time intervals of  $t_1, t_2, t_3, t_4, t_5 \dots$  from the beginning of sedimentation, then the change in the meniscus during the various time intervals will have been brought about by the settling of the particles as follows:

$$(a) \quad A + B \times \frac{t_1}{t_2} + C \times \frac{t_1}{t_3} + D \times \frac{t_1}{t_4} + E \times \frac{t_1}{t_5} \dots \text{after time } t_1$$

$$(b) \quad A + B + C \times \frac{t_2}{t_3} + D \times \frac{t_2}{t_4} + E \times \frac{t_2}{t_5} \dots \dots \text{after time } t_2$$

$$(c) \quad A + B + C + D \times \frac{t_3}{t_4} + E \times \frac{t_3}{t_5} \dots \dots \text{after time } t_3$$

$$(d) \quad A + B + C + D + E \times \frac{t_4}{t_5} \dots \dots \text{after time } t_4$$

$$(e) \quad A + B + C + D + E \dots \dots \text{after time } t_5$$

Or the change in the meniscus, say, during the time interval between  $t_4$  and  $t_5$  has been brought about by the settling of  $E\left(1 - \frac{t_4}{t_5}\right)$  fraction of particles of diameter  $d_5$ . Or the percentage of particles of diameter  $d_5$  is simply determined by dividing the change in the meniscus reading between times  $t_4$  and  $t_5$  by  $\left(1 - \frac{t_4}{t_5}\right)$ . Similarly the meniscus changes due to the settling of other particles are computed. Since the total change in the meniscus reading due to the settling of the whole suspension is known, the percentage of any fraction is easily calculated.

Times  $t_1$ ,  $t_2$ , etc. corresponding to diameters  $d_1$ ,  $d_2$ , etc. are calculated from Stokes' law or may be taken from the table given by Puri and Amin (8).

#### DIRECTIONS FOR FILLING THE INSTRUMENT

The density of aniline-benzene mixture is adjusted by the help of a hydrometer. This approximately corresponds to 4 parts of aniline and 1 part of benzene. About 500 cc. is prepared and stored for use. It is to be remembered, however, that since aniline-benzene mixture has a coefficient of expansion higher than that of water, the density should be adjusted whenever there is an appreciable rise or fall in the working temperature, especially the former.

The apparatus is thoroughly cleaned with  $H_2SO_4$ -chromic mixture before filling. This is done best by filling the manometer through the middle vertical tube with the cleaning mixture and leaving overnight. Next day, after thorough washing, the instrument is filled with water to which just enough NaOH has been added to make it alkaline to phenolphthalein. The vertical tube is then filled with the aniline-benzene mixture through the cup. The side tubes are then put into two cylinders filled with water to the same level and connected through the manometer by gently opening the tap connecting the vertical tube with the manometer. When the appropriate amount of the mixture has been let in some mercury is poured into the vertical tube, and by again opening the tap gently it is allowed to rise about a centimeter above the tap. This mercury seal is necessary, as otherwise the aniline-benzene mixture dissolves the tap grease and slowly creeps out. The actual time taken in filling after cleaning the instrument is not more than 10-15 minutes.

The meniscus in the manometer should move up and down freely with an even convex curvature. If any tendency to stick or flatten at the interface is noticed, it is an indication that the instrument has not been cleaned properly. Sometimes the aniline-benzene thread breaks or shows a tendency to break, in that case the whole of it is swept out by dipping one of the side tubes into a cylinder containing alkaline water and syphoning. Mercury is then poured out. Bubbles of air that collect in the spiral tubes as a result of the pushing out of mercury are removed by syphoning out water and refilling the manometer as before. This emptying and refilling takes only 5 minutes at the most.



The cylinders should be kept over a stone bench. Slight variations of temperature as long as they affect the two cylinders equally do not matter, but if one side faces a window, or the room is draughty, the results might be affected, in which case the cylinders might be kept in bottomless wood cases or immersed in a tank of water.

#### EXPERIMENTAL

Fifty grams of soil were treated by the NaCl-NaOH method of dispersion described by the author elsewhere (6), made up to 2,500 cc. (2 per cent suspension), and transferred to the sedimenting cylinder. Into another cylinder, 2,500 cc. of water having the same temperature as the suspension was poured and the two were kept side by side. Immersing the instrument to 30 cm.

TABLE 1

*Mechanical analysis of two soils by the manometric and pipette method*

DIAMETER OF PARTICLES	SUMMATION PERCENTAGES			
	P C. 1		P.C 2	
	Pipette	Manometric	Pipette	Manometric
<i>mm.</i>				
0.06	83.6	85.5	89.6	..
0.05	.	79.2	....	91.5
0.04	71.4	71.5	86.5	89.6
0.03	....	63.0	. .	86.2
0.02	54.2	52.8	78.4	81.7
0.01	38.6	38.2	70.2	71.9
0.008	35.3	34.0	67.8	68.6
0.006	31.4	30.2	65.1	65.2
0.005	..	27.8	. .	62.3
0.004	26.6	25.9	62.2	62.2
0.002	21.8	22.9	59.3	59.4

depth enabled the first reading to be taken when all particles larger than 0.05 mm. diameter had settled down. Further readings were taken at suitable intervals of time corresponding to other diameters according to the working temperature. For this purpose the table of settling velocities at different temperatures given by Puri and Amin is used (8). After the larger particles, say up to 0.01 mm. diameter, have been determined, the suspension is shaken again and the instrument let down to a depth of 15 or 10 cm.; this reduces the time intervals to one-half or one-third respectively, with a corresponding decrease in magnification.

When the suspension has been settling for a few hours and it is necessary to find the percentage of particles left in it, the instrument is gently raised, after the two-way tap has been closed, by 1 or 2 cm., and the change in the manometer noted after the connection has been established again.

Since

$$D^2/d^2 \times (S - 1) v/200 = a$$

where  $S$  is the density of the suspension, if now the instrument is raised by 1 cm. we have

$$D^2/d^2 \times (S - 1)(v - 1)/200 = a'$$

and the change in the manometer reading being  $a - a'$ , we have

$$\begin{aligned} a - a' &= D^2/d^2 \left( \frac{(S - 1)v - (S - 1)(v - 1)}{200} \right) \\ &= D^2/d^2 (S - 1)/200 \end{aligned}$$

From this equation  $S$  can be calculated, and being proportional to the concentration, the latter is easily known. In practice the relation between  $a - a'$  and  $S$ , or rather concentration of suspension, is worked out once for all as a part of the calibration of the instrument.

In table 1 are recorded the results of mechanical analysis of two soils P. C. 7 and P. C. 2 by both the manometric method and the pipette method using 2 per cent suspension in every case. As will be seen, the agreement is quite good.

#### SUMMARY

A manometric apparatus for the mechanical analysis of soils is described. It is based on the principle of the differential liquid manometer, having a mixture of aniline and benzene (of density slightly greater than water) as the heavier liquid and water itself as the lighter liquid.

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# LYSIMETER STUDIES: I. MOISTURE PERCOLATION THROUGH THE SOIL PROFILE<sup>1</sup>

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The movement of moisture through the soil under natural conditions has not been studied to any extent because of the difficulties involved in retaining the undisturbed state of the soil mass during the process of experimentation. Most of the studies on the movement of the accepted conventional forms of water—gravitational, capillary, and vapor—were made on soil material. Green (6) justly remarks that in order to get a true picture of the moisture properties of the soil, a technique has to “be developed for measuring the soil characteristics *in situ*; until then laboratory experiments will deal not with soils, but with soil materials.”

## REMARKS ON LYSIMETER STUDIES

An approach to the study of the movement of moisture under natural conditions was attempted as early as the end of the eighteenth century by the introduction of the so-called drain-gauges, later known as lysimeters. Dalton's<sup>2</sup> original idea was to follow up the fate of the natural precipitation with reference to the feeding of ground waters which nourish the streams. One of the fundamental questions was then to find out how much of the precipitation percolates through the soil. Gradually the lysimeter method was adopted in studies of soils as such and the percolation or movement of moisture became a matter of secondary consideration. Analyses of the leachings in relation to the ammonia, sulfur, and other constituents lost from the soil and those brought down by the rain water became the primary purpose of lysimeter studies. Later and in recent years lysimeters have been also utilized for the study of the losses of fertilizer ingredients and soil amendments under cropped and fallow conditions. Moisture losses were of course considered in this connection.

And now, after close to 150 years of work with lysimeters, we are still in doubt about the nature of the movement and percolation of water through the soil—the phenomenon which was the fundamental cause of the origin of lysimeters. Lysimeters are still open to the serious criticism, which has been

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

<sup>2</sup> Dalton the famous English scholar, the father of the atomic theory, is credited with being the first one to install lysimeters. A more detailed discussion of the origin, history, and development of lysimeters is now in the process of preparation.

recognized ever since the early days of lysimeter studies, namely, that the findings do not represent the true conditions of the soil; they are true for the *one or the other soil material prepared and arranged in a particular way*. And there are countless ways of preparing and arranging the soil material and each arrangement will give a different answer—or at most not all of them will give the same answer—to the problem under consideration. For there is only one way in which the soil will respond as a soil and that is when it is not disturbed. Only then may one study its behavior and reactions, when its natural arrangement, its constitution, which has formed in the course of ages, its structure, and its make-up are still preserved and in place.

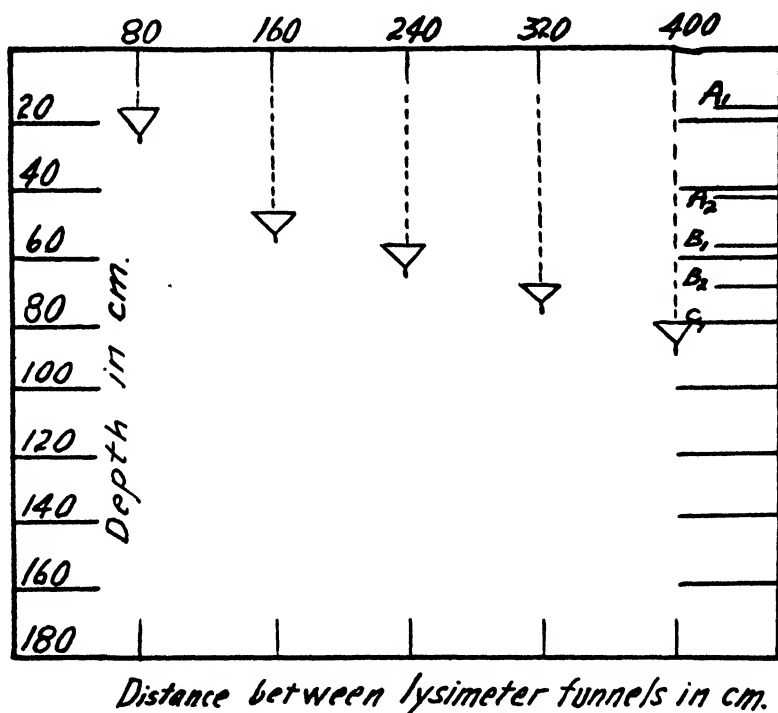


FIG. 1. DIAGRAMMATIC SKETCH OF LYSIMETERS UNDER RESPECTIVE HORIZONS

No botanist would study plant reactions with respect to the behavior of the plant as a whole by macerating the plant. And it is just the thing we are doing when we dig up the soil body, remove stones, sieve the material, and dump it into lysimeter tanks. By this method not a single element of the natural conditions of the soil body is preserved, save the rainfall; and even this natural factor is frequently interfered with. It is known that artificial watering of the lysimeters is sometimes resorted to. In some lysimeters the rims are arranged to prevent surface run-off—another abnormal condition.

Anyone familiar with the fundamentals of the genetic school of soil science appreciates what it means to take out the soil material foot by foot, mix it,

and place it into a tank in the "same order." Actually it means to upset the established conditions, mix some of the material from the horizon of illuviation with that of the horizon of eluviation. This of course will introduce an entirely new set of physical, chemical, and biological reactions, since each of the horizons maintains a definite set of these reactions true to itself and not true for any other horizon. It is, for instance, very probable that in the Cornell Agricultural Experiment Station lysimeters (11, 12) the first foot of soil placed on top contained the surface of the B<sub>1</sub> horizon. The data (11, p. 11) on the mechanical composition of the soil used in these lysimeters show that the first foot contained 22.63 per cent of clay, the second foot—32.72 per cent, the third foot—38.62 per cent, and the fourth foot—36.67 per cent. It is apparent that the second foot of the soil, with 50 per cent more clay than in the first foot, represents the B horizon which extends apparently through the entire or a part of the third foot. Under such conditions some of the colloidal substances from B<sub>1</sub> would tend to produce a compact, impervious layer close to the surface and prevent the percolation of the rain water. Very few of the inherent soil forming elements and reactions of the A horizon, such as porosity, texture, structure, organic matter content, and aeration could behave normally. It is therefore no wonder that in the uncropped lysimeters of the Cornell experiment station the soil "*became so compact that at the end of ten years, drainage was poor and water frequently stood on the surface for considerable periods,*"<sup>3</sup> (12, p. 6). Of course, cropping the soil will tend to break up the surface compact layer and the introduction of the organic matter will bring into play the eluviation reactions and hence improve the leaching capacity of the soil material.

The aforesaid might also explain the "peculiar" behavior of the tanks with respect to the nitrogen balance (12, p. 38):

In 1914 the nitrogen showed a tendency to fall below its usual ratio to the flow of drainage water. It is difficult to trace this to any weather conditions obtaining that year, as neither the rainfall nor the temperature was abnormal during the months when nitrate formation might be expected to be active. It is possible that with the settling of the soil in the tanks and the diminished aeration, there is a tendency for the process of nitrate formation to be less active.

In the lysimeters of the Geneva station (New York State Agricultural Experiment Station) the soil material was placed into the tanks in a somewhat different order from that in the Cornell lysimeters.

The bottom 2 feet of the 4-foot tanks received soil from the 16–36 inch depth. The second foot received soil from the 8–16 inch depth, while the surface 8 or 9 inches received soil from the 0–8 inch depth.

The soil material is of a lighter texture than the soil in the Cornell lysimeters.

<sup>3</sup> Italics are mine.

Still in the Cornell lysimeters 78 to 82 per cent<sup>4</sup> of the total rainfall percolated through the unplanted tanks and 54 to 62 per cent through the planted tanks, and the Geneva lysimeters lost only 40 per cent of the total rainfall from the fallow tanks and 21 per cent from the planted tanks. Thus the Geneva figures are only about one-half those of the Cornell lysimeters. And still the total rainfall and the temperature are about the same in the two localities. Collison and Mensching (1) from the Geneva station find it difficult to explain such great discrepancy. It is the opinion of the author that the abnormality may be laid to the abnormal conditions of arranging the soil material in the tanks.

In the Tennessee lysimeters, Mooers (17) filled his tanks by following the Cornell (11) method, except that the surface 6 inches came from the plowed layer. Each layer "after being carefully mixed was laid down in the same order in the can as it was removed from the field." The tanks are  $\frac{1}{5,000}$  of an acre; there are tanks of various depths, from 6 feet to 1 foot, the latter having only 9 inches of (surface ?) soil material.

MacIntire (13), also from Tennessee, installed another set of tanks embracing an area of  $\frac{1}{1000}$  of an acre. Two depths were used: 12 inches and 24 inches. The soil

was thoroughly screened through a  $\frac{1}{4}$ -inch sieve, carefully mixed and closely covered during the overnight period required for the making of moisture determinations, and during the mixing of the soil and the materials. One hundred pounds of moisture-free surface loam soil representing a depth of approximately 8 inches was used for each tank.

In the 2-foot tanks the surface 8 inches was underlaid with a foot of clay subsoil. Thus these lysimeter tanks differed somewhat from Mooers' inasmuch as they contained soil material from below the plowed layer, which is usually not more than 5 to 6 inches deep. From the data presented by MacIntire (14, p. 383) on the leachings of six tanks without and six tanks with a subsoil and the rainfall data of the meteorological station at Knoxville, in the vicinity of which the lysimeters are located, it has been calculated that over a 4-year period the average amount leached was about 44 per cent of the total rainfall. And these were *uncropped tanks* but treated with lime. Two-year data presented by Mooers (18, p. 131) on the leachings from 4-foot lysimeters on three soil series show about the same (43.12, 50.86, and 42.50 per cent respectively for the three series of soils) per cent of leachings. And the 4-foot tanks were *cropped*, a condition which as a rule cuts down the percolation of moisture.

Why both types of lysimeter tanks at Tennessee should percolate practically the same amount of leachings and incidentally be about equal to the amount

<sup>4</sup> In the recent publication from Cornell (12) the figure for the fallow tanks is 66 per cent. The discrepancy is due to the peculiar method of averaging the results of the fallow tanks. The original fallow tanks were cropped after the first 10 years and other tanks which were cropped until then were fallowed for 5 years and the leachings from these were averaged with those from the original fallow tanks. These "newly" fallowed tanks percolated less water and in that way the average was reduced from 82 to 66 per cent.

leached from the Geneva lysimeters is a matter of conjecture. The climatic conditions in the two localities vary considerably; the soil material used, the depths from which it was dug, and the way it was placed in the lysimeter tanks in the two stations, all were different. The only equality factor in the two sets of lysimeters is the abnormal condition of the soil as such, i.e., there is no soil body in either of the lysimeters. Another similarity might be found in the fact that the surface soil material was probably from the A horizon. Some abnormalities about lysimeters have been recognized by those responsible for installation. Thus Mooers (19, p. 10) states:

The data from the cylinder experiments, as published in Bulletin 118, show that soils placed in galvanized iron cylinders and lysimeters and exposed to the weather, behaved in a decidedly abnormal manner as compared with soils in the field.

The source of these abnormalities was not sought in the make-up of the soil body which was destroyed by "carefully mixing" the soil material as it was dug out from the field. Some other external factors were made responsible, one of which was the "lack of run-off caused by the projection of the rims for two or three inches above the soil."

In view of the aforesaid, the type of lysimeters used at Cornell, Geneva, Tennessee, and other experiment stations and agricultural institutions is not suitable for the study of the movement of moisture through the soil. The data obtained from the leachings of such lysimeters cannot be applied to interpret phenomena and reactions that might occur under natural conditions in the field. What one might expect from lysimeters placed in the soil body will be shown in the following data; but before these data are presented a review will be made of a note by Mooers and MacIntire (20) in which an argument is raised about the author's claim (17) that "the only rational method of studying the drainage of each horizon is to install a series of lysimeters at the depths of the respective horizons without disturbing the natural position of the soil profile." They claim that "if this unqualified statement be true it means that during the past twenty years *the properties of the several profiles of different soils*<sup>5</sup> have been studied by an irrational method at the Tennessee Station, and with three types of equipment."

An examination of the many papers published by Mooers and MacIntire and associates revealed no lysimeter studies involving "*the properties of the several profiles of different soils.*" From the publications mentioned it is clear that the lysimeters installed in Tennessee were filled with soil material with no regard to the profile constitution of the soil body. It is thus apparent that as far as the study of the soil as such is concerned *the method used is irrational.*

Another point raised by Mooers and MacIntire is that "homogeneity in the soil mass is the paramount essential in lysimeter experiments." This phase has been dealt with by MacIntire elsewhere (15), but from neither one of the publications is it clear just what is meant by "homogeneity in the soil mass."

<sup>5</sup> Italics are mine.



It is generally understood that the soil is not a homogeneous system, which by the definition, as applied in the "Phase Rule" (4), is one "which is uniform throughout its whole extent and possesses in every part identical physical properties and chemical composition." And it is therefore also puzzling why Mooers and MacIntire (20) attributed to the writer the thought that "homogeneity is hoped for or assumed" in the new type of lysimeters installed at the New Jersey Agricultural Experiment Station. On the contrary, from the genetic point of view in soil science the soil as an independent natural body with a clear-cut separation of horizons in the profile<sup>6</sup> is a heterogeneous system which, by definition (4), "consists of parts which have different physical properties, perhaps also different chemical properties, and which are marked off and separated from one another by bounding surfaces." Although this definition as applied in the "Phase Rule" may not at present be applicable in its entirety to the soil system, it approaches it and with the broadening out of the physico-chemical principles it might in time be applied fully. At any rate the principle of homogeneity is ruled out from the soil system.

The argument raised by Mooers and MacIntire (20) that "the value of the lysimeter is that it serves as a means of establishing fundamental principles that relate to cultivated soils and their subsoils" is not convincing. For after all, cultivated soils are nothing more than natural soils in which the upper 5 to 6 inches (in some cases slightly deeper) are disturbed. Beyond these 5 to 6 inches, which as a rule are a part of the horizon of eluviation, the soil body remains undisturbed and influences the behavior of the overlying cultivated soil material. To take this material from a depth of 5 to 6 inches, confine it into a cylinder, and make it 9 inches deep or more, means the introduction of a new set of conditions: moisture, temperature, and pressure relationships change and with them other physical, chemical, and even biological properties change.

The question raised by Mooers and MacIntire (20) about the perpendicular movement of moisture in the soil is, from the data obtained by the new type of lysimeters, answered in the negative. No claim has been made by the author that the direction of the gravitational water is perpendicular, as might be implied from the statement by Mooers and MacIntire. Just because moisture does not move exclusively in a perpendicular direction, there is more reason to criticize the filled-in type of lysimeters which force the rainfall to follow a perpendicular direction.

The underlying motive for the installation of lysimeter equipment is the study of the drainage, which in turn might tell us about the movement and translocation of the soil and plant-food constituents which are formed in the soil profile or which are added naturally by the humus-accumulative Ao layer or through the addition of fertilizers in the cultivated soils. And for this reason the method with the filled-in type of lysimeters is irrational and the findings from such data cannot be applied to soil conditions. The leachings from such

<sup>6</sup> In a mature soil the morphological, chemical, and physical characters are distinct; in an immature soil the chemical and physical properties differentiate the horizons in the profile.

lysimeters give no picture of what is going on in the soil, for they do not contain the heterogeneous soil body. They might give an inkling about some reactions that take place in the soil material under this and no other condition and, by the way, each one of such strictly controlled conditions has many uncontrollable factors—and will not hold true with the slightest change made.

Most of the data obtained with the filled-in type of lysimeters could just as well be procured by conducting similar experiments in drainage pots under controlled conditions in the greenhouse and in the laboratory. As long as the fundamental requirement of having the soil in a natural state cannot be satisfied by the filled-in type of lysimeter, there is no point in preferring an out-of-door elaborate and expensive system of drainage tanks to one in the greenhouse. There is the remote possibility of utilizing the filled-in type of lysimeters for the study of soil formation. In the course of time the soil material in the tanks will undergo changes due to the activity of the soil formers. A soil body with a differentiation of horizons will be created. But it seems that it would be a rather long drawn out experiment and, besides, we have at our disposal simpler and well-proved methods for the study of soil formation.

The criticism and analysis advanced against the filled-in type of lysimeters of the Tennessee, New York, and Cornell experiment stations apply as well to others. To overcome some of the cardinal objections raised, a new type of lysimeter has been installed at the New Jersey Agricultural Experiment Station. It was realized at the outset that in connection with this new type of lysimeter we must take into consideration the uncertainty about the true quantitative factor with regard to the percolation, of which more will be said in the course of presenting the data for the first 2 years. Other minor factors which might introduce some complications in applying the data of the leachings to actual natural conditions in the soil will also be brought out. On the whole, however, the data obtained give very striking and illustrative information as to the movement and translocation of the moisture and the substances carried in it through the soil profile.

#### NEW TYPE OF LYSIMETERS

A pit is dug 120 cm. wide, 180 cm. deep—depending on the profile depth of the soil studied—and 460 to 500 cm. long. Under each horizon a tunnel, 50 cm. long, is dug in the shape of the lysimeter which consists of a shallow funnel made of block tin 30.6 cm. in diameter ( $\frac{1}{55,000}$  of an acre in area), 4 to 5 cm. deep, with a sharp edge 3 to 4 mm. high, and with nine to ten 2-mm. perforations in the neck. This funnel is filled with quartz pebbles and its sharp edge rests against the roof of the tunnel. When the funnel is placed in the tunnel the distance from the wall of the pit to the funnel is 20 cm. The funnel is wedged upward and the open space between it and the wall of the pit is filled with the soil material dug out from the tunnel. After all the funnels are placed under the respective horizons—80 cm. apart on a horizontal line as

shown diagrammatically in figure 1—a board wall is built 8 to 10 cm. away from the soil wall and the space is filled in with soil. Reinforcing and bracing the studdings to which the wall boards are nailed is an important precaution against their collapsing under the weight of the filled in soil.

Each funnel is connected by means of a coupling with a block tin tube 6 mm. in internal diameter. The tube leads to a receptacle for the leachings. Figure 1 gives the front view of the wall with the lysimeter funnels in place under the respective horizons.

The pit is covered with a shed roof extending just a few centimeters above the ground. A gutter leads the water off to a distance away from the area where the lysimeters are located.

Plate 1, figure 1, gives a general view of the location of the lysimeter pit and its general external appearance and surroundings. Figures 2 and 3 of plate 1 show the interior of the lysimeter pit. Attention is called to the fact that the points where the tubes come out from the walls—as shown by the tape and letters—do not indicate the exact position of the funnels. The tubes were bent downward and the angle was not the same for all the funnels.

The new type of lysimeter is rather old. Gemmerling (5) designed his after the pattern of Ebermayer (2, 3), who used this type of lysimeter in studies on the moisture relationships in the forests. In his studies on the methods of investigating soil moisture, Popov (21) also used this type alongside a removable type.

A virgin forest soil mapped by the Soil Survey as Sassafras sandy loam was selected. Morphologically this soil shows feeble signs of podzolization, except perhaps some specks of  $\text{SiO}_2$  in the  $A_2$  horizon, which has a slightly lighter grayish brown tinge than the overlying  $A_1$ . The depth of the respective horizons as determined by the standard methods of profile study is indicated on the diagram. Chemically<sup>7</sup> this soil shows distinct podzolization and is to be classified as a podzolic, or slightly podzolized soil, a subgroup of the large zonal type—the podzols.

The tree vegetation<sup>8</sup> on the lysimeter plot consists of: black locust (*Rubinia pseudo-acacia* L.), flowering dogwood (*Cornus Florida* L.), sassafras (*Sassafras variifolium* [Salisb.] Kuntze), white oak (*Quercus* sp.), black oak (*Quercus* sp.), red maple (*Acer rubrum* L.), shag-bark hickory (*Carya ovata* [Mill.] K. Koch), and sweet cherry (*Prunus avium* L.). The trees are listed in the approximate order of abundance.

One property of the soil on the plot must be mentioned at this time, namely, the moisture content. The material was sampled just when the lysimeters were installed and the soil seemed to have been approximately in its optimum moisture condition. The figures given are on the basis of the volume-weight

<sup>7</sup> A complete chemical analysis of soil by horizons will be presented in the next paper of this series.

<sup>8</sup> For this information the author is indebted to Dr. C. W. Watson, National Research Fellow at the New Jersey experiment station.

of the soil.  $A_1$  contained 30.23 per cent moisture,  $A_2$ —24.26 per cent,  $B_1$ —27.8 per cent,  $B_2$ —16.7 per cent, and  $C_1$ —15.2 per cent.

#### SAMPLING AND ANALYZING LEACHINGS

The leachings, if there are any, of each horizon are being collected after each rain. Each leaching is measured; conductivity—by the Kohlrausch method—and pH—by the colorimetric method—are determined soon after it is brought to the laboratory. An aliquot is taken for analysis of dry matter, organic matter, and mineral constituents. Another aliquot is taken for total nitrogen. In addition, nitrates, sulfates, and chlorides are determined on separate aliquots.

The analyses mentioned are made whenever there are enough leachings. Otherwise the conductivity, pH, nitrates, sulfates, and chlorides only are determined.

#### LYSIMETER LEACHINGS FOR THE FIRST 2 YEARS

##### *A<sub>1</sub> horizon*

The lysimeters were installed in the middle of June, 1929, and the first leachings appeared June 22. The data on the leachings that percolated through the  $A_1$  horizon for the two leaching years (the year is figured from June 22, the date when the first leachings appeared) are presented in table 1.

It will be noted that during the year 1929–30 with 37.26 inches of total rainfall 21.00 liters of leachings were collected from the lysimeter funnel under  $A_1$ , whereas during the year 1930–31 with 34.14 inches of rainfall 29.223 liters were collected. If we calculate<sup>9</sup> the leachings on the acre basis we find that in 1929–30 out of the 135,253.8 cubic feet of total precipitation only 40,771.5 cubic feet percolated through the  $A_1$  horizon, a depth of 18 cm.—about 7.5 inches—or 30.14 per cent. In 1930–31, out of a total of 123,928.2 cubic feet of precipitation 56,736.5 cubic feet, or 45.7 per cent, percolated through the  $A_1$  horizon. Thus 50 per cent more—the difference between 30.14 and 45.7 per cent—of the total rainfall percolated through  $A_1$  in 1930–31 than in 1929–30. Actually, however, only 39.1 per cent—the difference between 21.0 liters in 1929–1930 and 29.223 liters in 1930–31—more water percolated. And yet the total rainfall for 1930–31 was 9.1 per cent lower than in 1929–30. Apparently the amount of percolation does not depend altogether on the total rainfall, but also on some other conditions. What these are has not been as yet fully established.

<sup>9</sup> The calculation is made as follows: The total leachings for the year are converted into cubic feet by multiplying the number of liters by 0.0353—the factor for conversion of liters into cubic feet. To obtain the total leachings on the acre basis, the cubic feet per lysimeter are multiplied by 55,000, the area of the lysimeter funnel being  $\frac{5}{8} \times \frac{1}{8} \times \frac{1}{8}$  of an acre. To get the total rainfall on an acre basis the area of an acre—43,560 square feet—is multiplied by  $\frac{1}{12}$  (an acre inch) and then by the number of inches of rainfall for the year.

From observations for the 2 years and from theoretical considerations, it has been inferred that the state of the colloidal complexes in the soil to a certain extent controls the rate of percolation.

*Capillary and non-capillary pore space.*—When the soil is subjected to a prolonged drought the colloids—the inorganic and organic, especially the latter—lose their water of hydration, dry, and shrink. The individual textural units become cemented into structural units, decreasing thereby the capillary pore space and increasing the non-capillary pore space. And it is the latter that facilitates percolation. When, however, the soil becomes saturated the colloids imbibe water, become hydrated, and swell. A disintegration of the structural units, or, as it is sometimes called, “slaking,” takes place and the result is an increase in capillary pore space and decrease in non-capillary pore space. This process hinders percolation.

Observations on the rate of percolation through the  $A_1$  (the same holds true also for  $A_2$  and the other horizons) horizon for the 2 years of lysimeter investigations have invariably proved that after prolonged droughts more leachings came through with relatively low amounts of rainfall, whereas during periods of moist conditions of the soil the amount of leachings was low even with a relatively high rainfall. An analysis of the rainfall data presented in table 2 in correlation with the data on the leachings as given in table 1 will illustrate the aforesaid.

After the heavy rain of 1.71 inches on June 21, which was preceded by a spell of hot weather, 1,065 cc. of leachings came through the  $A_1$  horizon. This was followed by 0.92 inch of rainfall on June 22, which yielded 510 cc. of leachings. After that it rained almost daily up to July 3, followed by 2 rainless days, and continued with intermittent rainy and rainless (more rainy) days up to July 14. During this period the soil was in a moist condition. The data in table 1 show that after the leachings from the rain of June 23 (0.92 inch) none came through even after such heavy rains as during July 5 and 6 (0.99 inch) and July 14 (0.69 inch). It is reasonable to assume that during this rainy period the non-capillary pore space decreased as a result of the hydration and swelling of the colloids. However, the 2 weeks of drought after July 14 changed the condition of the colloids in the reverse direction and the relatively low rainfall (0.66 inch) on July 29 and 30 yielded 1,400 cc. of leachings, almost three times as much as on June 24 after a rainfall of 0.92 inch—almost a third more than on July 29 and 30.

Two other striking examples illustrating the effect of drying on the rate of percolation may be found in the data on the rainfall for July, August, and October, 1930. From July 25 to August 23, a period of 3 weeks, a severe drought prevailed. The soil in the open fields adjacent to the woods where the lysimeters are located cracked, forming large structural aggregates. As a result of that the soil was very porous and the relatively small rain (0.44 inch) on August 15 yielded some leachings—80 cc. Again, in October, after a prolonged drought a rainfall of 0.65 inch on October 15 yielded 325 cc.

and, after the drought, toward the end of the month a rainfall of only 0.37 inch on October 29 yielded 608 cc.

Another element to consider in connection with the rate of percolation is the electrolyte content of the leachings. A high electrolyte content favors the coagulation of the colloids, which in turn decreases the capillary pore space. From data on hand, which will be published in the next paper of this series, it is evident that during the period of tree defoliation—usually in October and November—the electrolyte content of the leachings, as determined by conductivity measurements and actual analysis, is at its peak and, of course, the state of the colloids is affected.

*Significance of effective precipitation.*—The effective precipitation—the rainfall after which leachings appear—is one of the apparent and quantitatively measurable factors in the complex relationships between the rainfall and percolation. From the columns on the effective precipitation and the other data in table 1, it is evident that this apparent factor is independent of the total rainfall. During the first lysimeter year (1929–30) with a total precipitation of 37.26 inches there were 22.47 inches of effective precipitation. During the second year, with a lower total precipitation—34.14 inches, there was a higher effective precipitation—24.81 inches. Moreover, the data show that the higher the effective precipitation the higher is the percolation through the horizon under discussion. It is important to note that the amount of leachings is directly related to the effective precipitation, but the ratio differs for the different years. For the year 1929–30 the ratio of effective precipitation to leachings is 1.07 and for 1930–31 it is 0.849. Neither are the ratios of effective precipitation to total rainfall the same. For 1929–30 the ratio is 0.603 and for 1930–31 it is 0.726.

The lack of correlation between the total and effective precipitation is also brought out in table 1 in the columns on effective precipitation, per cent of total. The inequalities noted—lack of correlation between effective precipitation and total leachings and total precipitation—are undoubtedly due to the differential behavior of the soil colloids as outlined.

*Relation of temperature to percolation.*—That the mean monthly temperature *per se* is not a factor in the rate of percolation may be inferred from the data in table 1. For the 2 years under consideration the total accumulated temperatures were almost exactly the same and there were very small differences between respective months during these 2 years, and yet it is unquestionable that the temperature had an effect on the rate of percolation, for it is the temperature which is under some conditions responsible for the moisture relationships in the soil. It is the temperature in the intervals between rains that controls evaporation (humidity is linked up with it) and causes drying out of the soil, which in turn determines the capillary and non-capillary pore space, especially in the  $A_1$  horizon.

*Summary statement about the leachings through  $A_1$ .*—The outstanding feature about the percolation of rainwater through the  $A_1$  horizon is that notwithstand-

ing its shallowness—18 cm. deep—only 30.14 per cent of the rainfall during 1929–30 and 45.7 per cent during 1930–31 percolated. This leaves 69.86 and 54.3 per cent, respectively, for evaporation from the surface of the soil and utilization of moisture by the plant cover. No evaporation data are available and therefore there can be no estimate of the amount of moisture available

TABLE 1

*Precipitation, leaching, and temperature data in connection with lysimeter studies on the A<sub>1</sub> horizon at a depth of 18 cm.*

1929-30							1930-31						
Laboratory number of leaching	Date of collection	Effective precipitation*	Total precipitation for month	Effective precipitation, per cent of total	Leachings	Mean monthly temperature	Laboratory number of leaching	Date of collection	Effective precipitation*	Total precipitation for month	Effective precipitation, per cent of total	Leachings	Mean monthly temperature
	1929	in.	in.	per cent	liters	°F.		1930	in.	in.	per cent	liters	°F.
1	June 22	1 71	...	...	1 065	...	16	July 10	1 46	...	...	1 300	...
2	June 24	0 92	...	...	0 510	...	17	July 23	1 65	4 08	76.22	1.300	75 2
3	July 30	0 66	2 94	22 45	1 400	73 7	18	Aug. 16	0 44	...	...	0 080	...
4	Aug. 15	1 31	2 41	54 36	0 755	70 8	19	Aug. 24	2 26	2 88	93 74	3 350	71 8
5	Sept. 9	4 07	...	...	4 740	...	20	Sept. 17	0 89	1 43	62 23	0 350	70 4
6	Sept. 18	0 91	5 59	89 08	0 470	68 0	21	Oct. 16	0 65	...	...	0.325	...
7	Oct. 3	2 10	...	...	3 440	...	22	Oct. 29	0 37	1 77	57 62	0.608	52 6
8	Oct. 23	1 06	4.11	76 88	0 930	53 4	23	Nov. 6	1 20	...	...	1 455	...
9	Nov. 5	0 94	...	...	0 390	...	24	Nov. 19	1 72	3 27	89 30	2 420	44.2
10	Nov. 20	1 05	2 21	90 04	0 135	45.0	25	Dec. 30	1 62	2 40	67 50	4 090	33 8
11	Dec. 20	1 35	2.93	46 07	1 180	33 4	1931						
	1930						00	Jan.	0.00	2 24	0 00	0 000	32 2
00	Jan.	0.00	2 65	0 00	0 000	31 8	26	Feb. 19	1 95	2 37	82 27	4 100	33 8
12	Feb. 18	1.70	3 00	56.66	3.435	36 2	27	Mar. 9	1 09	...	...	1 770	...
13	Mar. 11	1.34	2 31	58 00	0 250	40 5	28	Mar. 30	1 60	3.49	77 07	3 305	39 6
00	Apr.	0 00	2.18	0 00	0 000	48 2	29	Apr. 2	0 69	...	...	0.665	...
14	May 17	0 90	2 54	35 43	0 100	63 6	30	Apr. 12	0 73	2.37	60 00	0 505	50 7
15	June 11	2.45	4 39	55 80	2.200	72 0	31	May 15	1 65	...	...	0 700	...
...	...	...	...	...	...	...	32	May 24	0.68	3 24	71.99	0 475	61.4
...	...	...	...	...	...	...	33	June 11	2 62	...	...	1.950	...
...	...	...	...	...	...	...	34	June 18	1.34	4 60	...	0.475	70 4
Total for year.		22 47	37.26	...	21.000	636.6	...	...	...	34 14	...	29 223	636.1

\* By effective precipitation is understood the precipitation after which leachings percolated through the horizon.

to the plants. And besides, the moisture in this horizon is not the only source of water supply for the plants. A large share of the 30.14 and 45.7 per cent of rainfall that percolated through A<sub>1</sub> remained in A<sub>2</sub>, as will be shown presently, and this moisture is undoubtedly available to the plants.

There is another factor to consider in the calculations made on the water

percolation through the soil under forest conditions, and that is the amount of moisture that is retained by the forest canopy. It is well known that the fine rains do not reach the forest floor, as the leaves retain much moisture. Ebermayer (3) points out that coniferous forests keep back 40 to 50 per cent of the rainfall, and hardwood—10 to 25 per cent. Of course these figures will be greater or smaller depending on the thickness of the stand and the age of the trees. How much moisture is retained by the hardwood forest cover on the plot where the lysimeters are located has not been determined. Fifteen per cent would be a rough estimate. In that case the percentage of rainfall that percolated with respect to the total precipitation that reached the forest floor would be higher than the foregoing figures.

#### *A<sub>2</sub> horizon*

The data on the percolation of moisture beyond the depth of the A<sub>2</sub> horizon—42 cm.—as given in table 3, show that through this depth the amount of percolate was greater in 1929–30 than in 1930–31. Thus in 1929–30 only 7.93 per cent of the total rainfall percolated beyond the depth of the A<sub>2</sub> horizon and 7.1 per cent in 1930–31. It will be remembered that the reverse was true for the A<sub>1</sub> horizon notwithstanding the higher total rainfall during 1929–30.

If we take the amount of leachings that reached the A<sub>2</sub> horizon—21.0 liters in 1929–30 and 29.223 liters in 1930–31—and calculate how much of that passed through the A<sub>2</sub> horizon, we find that 5.525 liters, or about 25 per cent, percolated in 1929–30 and 4.275 liters, or 14.6 per cent, in 1930–31.

An understanding of the colloidal behavior of the soil will explain the apparent contradiction. As pointed out in the discussion on the percolation of moisture through the A<sub>1</sub> horizon, the rate of percolation is controlled by the relative abundance of capillary and non-capillary pore space, which in turn is determined by the intermittent drying and wetting of the colloids in the soil. In this respect there is less fluctuation in the A<sub>2</sub> horizon because the latter does not dry out so quickly as the A<sub>1</sub> horizon. Still this phenomenon of drying and wetting of the colloids manifests itself in the A<sub>2</sub> horizon and produces the same effect as in the A<sub>1</sub> horizon. During the months of the latter part of August, September, and October of 1930 the soil dried out beyond the depth of the A<sub>2</sub> horizon and consequently the non-capillary pore space increased. The rainfall during November was not effective enough to saturate the A<sub>2</sub> horizon. Only in December, after the soluble salts which accumulated in the A<sub>0</sub> layer because of the drought and which leached from the freshly fallen dead leaves had been washed down, was a rain of 1.62 inches so effective as to give as high as 1,870 cc. of leachings from the A<sub>2</sub> horizon. Observations made on a nearby soil profile cut have shown that only then did the soil in the A<sub>2</sub> horizon become saturated and remain moist.

An examination of the data on the effective precipitation in table 3 reveals the same tendency as in the A<sub>1</sub> horizon: a higher leaching is indicative of a higher amount of effective precipitation. Thus in 1929–30 there were 5.525



liters of leachings with an effective precipitation of 13.38 inches and in 1930-31, 4.275 liters with 7.60 inches of effective precipitation. The ratios of effective

TABLE 2  
*Rainfall data from day to day in inches\**

MONTH	DAY OF MONTH															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1929																
June						0.13		0.33						T.	0 37	T.
July	T.	0 28			0.44	0.51			0 25					0 69		
1930																
July	0.35	0 04	T.			0 20	T.		T.	1 46	0 02			0.12		
August									T.	T.					0 44	T.
October															0 65	T.

MONTH	DAY OF MONTH																TOTAL
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
1929																	
June	T.	....	T.	T.	1.71	T.	0 92	0 31	0 20	0 48		0 71	0 21	T.	....	5 37	
July	....	....	0 09	....	....	T.	0.02	....	..	..	..	....	0 27	0 39	....	2 94	
1930																	
July	....	....	....	....	0 02	1 65	0 03	0.19	T.	....	....	..	..	....	....	4 08	
August	0 01	0.17	....	....	....	....	2 26	T.	....	..	..	..	..	....	....	2 88	
October	....	0 14	..	..	....	..	..	T.	..	..	....	..	0 37	T.	0 61	1 77	

\* The rainfall data in this table and in the others were taken from "Climatological Data," United States Department of Agriculture Weather Bureau records of the meteorological station at New Brunswick, located within 150 feet from the lysimeters.

TABLE 3  
*Precipitation and leaching data on lysimeter under the A<sub>2</sub> horizon at a depth of 42 cm.*

1929-30				1930-31			
Laboratory number of leaching	Date	Effective precipitation	Leachings	Laboratory number of leaching	Date	Effective precipitation	Leachings
	1929	inches	liters		1930	inches	liters
1	June 22	1.71	0.165	25	Dec. 30	1 62	1.870
5	Sept. 9	4.07	2.400		1931		
7	Oct. 3	2.10	0.210	26	Feb. 19	1.95	1.200
11	Dec. 20	1.35	0.090	27	Mar. 9	1.09	0.250
	1930			28	Mar. 30	1 60	0 910
12	Feb. 18	1.70	1.265	34	June 18	1.34	0 045
15	June 11	2.45	1.395	..	.....	...	.....
Total for year.....		13.38	5.525	..	.....	7 60	4.275

precipitation to leachings are 2.42 in 1929-30 and 1.77 in 1930-31. It is of interest to note that during the 2 years the ratio of effective precipita-

tion to leachings is about twice as high in  $A_1$  as in  $A_2$  (2.42 and 1.07 for 1929-30 and 1.77 and 0.849 for 1930-31 respectively). *The importance of this ratio constant will be of greater significance if it should reoccur in the succeeding years.* Nothing can therefore be inferred at present from this interesting regularity.

### *B and C horizons*

The function of the B horizon in the soil profile is to "filter" out the substances which move from the overlying A horizon. This is especially true for the soils of the podzol zone, as shown elsewhere (8). Because of that the accumulative nature of the B horizon, known as the horizon of illuviation, is very definitely expressed in this type of soil. Ortstein, orterde, or the so-called various types of hardpan are encountered in the B horizon of these soils. One

TABLE 4  
*Precipitation and leaching data on lysimeter under  $B_1$  at a depth of 58 cm.*

1929-30				1930-31			
Laboratory number of leaching	Date	Effective precipitation	Leachings	Laboratory number of leaching	Date	Effective precipitation	Leachings
	1929	inches	liters		1930	inches	liters
5	Sept. 9	4 07	0 050	25	Dec. 30	1 62	0 160
	1930				1931		
15	June 11	2.45	0 050	26	Feb. 19	1.95	0 050
.	...	.	....	28	Mar. 30	1.60	0 025
.	..	...	...	33	June 11	1 35	0 040
..		.	..	34	June 18	1.34	0 020
Total for year. . . .		6 52	0 100	..	....	7.86	0 295

of the outstanding properties of this horizon is its relative imperviousness to water. With the advance in age and consequent maturity of such soils, the B horizon becomes more and more impervious. As a result of that the vertical movement of moisture becomes impeded and horizontal movement along this horizon sets in, a phenomenon to be reckoned with in lysimeter studies. This has been pointed out by Gemmerling (5), Kachinskii (9), Popov (21), and others.

In table 4 the leachings of  $B_1$  are presented. It is to be noted that no leachings appeared through  $B_2$ , although there were leachings from horizon C, as shown in table 5, and, what is more striking, twice as much as from  $B_1$ . Even if we assume that all of the leachings that went through  $B_1$  followed the vertical direction at the point where the funnel in the C horizon is located, there are still about 300 cc. of leachings unaccounted for. Undoubtedly some of the leachings followed the horizontal direction and penetrated the B horizon along root channels, cracks, burrows of insects, worms, and other animals.

The presence of various kinds of concretions in the B horizon may serve as indirect evidence that some of the leachings follow certain channels. Tube-like concretions along root paths and along the edges of crotovinas and many other types of concretions form in the B horizon, because the leachings from above, in their downward movement, strike these paths—the line of least resistance—and deposit on their way the substances which precipitate from solution. Eventually these paths become filled up giving typical concretions.

Why there were more leachings from B<sub>1</sub> in 1930–31 than in 1929–30 may be explained on the basis of the condition of the colloids, which are quantitatively higher in this horizon than in any other. Observations made during the fall of 1930 have shown the extremely dry condition of this horizon. Not much of the rainfall that penetrated through the A<sub>2</sub> horizon was available for the B horizon. Because of the dry condition the structural units increased in size, and cracks were apparent all through this horizon. The non-capillary

TABLE 5  
*Precipitation and leaching data on lysimeter in the C horizon at a depth of 80 cm.\**

1930–31			
Laboratory number of leaching	Date	Effective precipitation	Leachings
	1930	inches	liters
25	Dec. 30 1931	1 62	0.355
26	Feb. 9	1.95	0.080
33	June 11	1.35	0.075
34	June 18	1.34	0.100
Total for year.....		6 26	0.610

\* No leachings appeared during 1929–30.

pore space increased and any water that reached this horizon easily percolated through it. Only after December 30, 1930, did the soil in this horizon become saturated and just then a large amount—comparatively speaking—reached the bottom of it.

During this period more than 50 per cent of the leachings percolated through the funnel in the C horizon. And still no leachings appeared from the funnel under B<sub>2</sub>. This is undoubtedly due to the phenomenon already referred to, namely, that the moisture is, under certain conditions, not moving entirely in the vertical direction. Because of that there seems to be no consistent relation between the effective precipitation and leachings in either of the B or C horizons. In 1929–30 the ratio of effective precipitation to leachings for the B horizon is about 65, for 1930–31, about 26, and for C it is—in 1930–31—about 10. One might expect a more consistent relation in the C horizon because it is not subject so much to possible horizontal movement of moisture. Its physical and chemical make-up—more or less constant moisture content,

no alternate drying and wetting, fewer colloids—precludes the possibility of much horizontal movement. Another point to remember is that the moisture content of the soil material in this horizon is lower than that of B and will therefore retain less moisture.

Before anything more definite may be said about the nature of the movement of the moisture through B and C, more data—2 to 3 years—will have to be accumulated.

#### GENERAL DISCUSSION

From the data and discussion presented, certain generalizations about the movement of moisture through the soil profile may be made. First of all, it is apparent that only a small fraction of the rainfall disappears in the form of ground water. If we take the percolation through C as a criterion and consider it as the moisture that goes to feed the ground waters, we find that no moisture was available for the ground waters in 1929–30 and only about 1 *per cent* in 1930–31. We must bear in mind that 2 years' results are not sufficient to warrant definite conclusions, especially since the rainfall was below normal in both of these years—10.0 inches in 1929–30 and 13.12 inches in 1930–31. It remains to be seen what the next few years will show.

The next point to be noted is that ordinarily there is not much, if any, horizontal movement of moisture through the A horizon. This makes it possible to assign quantitative significance to the data on the leachings. Quantitative data on the translocation of the substances in the leachings through the profile are very important, although even the qualitative aspect of the data gives a definite picture as to what is going on in the natural soil, as will be shown in the forthcoming papers of this series.

From the agronomic point of view the data on the amount and depth of penetration through the A horizon are very illuminating. A single example may suffice to illustrate this point.

Crop records from this station<sup>10</sup> show that the yield of corn—taking an average of four varieties—in 1929 and in 1930 was 57.4 and 56.9 bushels an acre respectively. The stover yield, however, was 7,826 pounds in 1929 and 12,157 pounds in 1930. The figures in table 1 show that during July, 1929, only 22.45 per cent of the total precipitation was effective, i.e., it penetrated through A<sub>1</sub>, whereas during the same month of 1930 the effective precipitation amounted to 76.22 per cent. And it is during this period—before tassling, from July 10 to August 1—that the corn plant makes its vegetative growth. It is reasonable therefore to ascribe the wide variation in yield of stover to the difference in effective precipitation.

An important point to consider in lysimeter studies is the behavior of gravitational moisture in columns of soil material. It has been shown by Lebedev (10) that a short column of sand or soil material will contain more moisture

<sup>10</sup> The author is indebted to Dr. H. B. Sprague, agronomist of the station, for the crop data furnished.

than a long one at certain points at the bottom of the respective columns. He has also shown that short columns of sand or soil give less leachings than the long ones. This raises the question of the percolation of leachings through the columns of soil represented by the various horizons. Of course the columns have to be in a state of saturation and, as may be judged from the data in tables 1, 3, 4, and 5, there was probably no instance of complete saturation of the entire soil column up to the C horizon. We can well imagine the  $A_1$  horizon becoming saturated, and in this case the objection raised by Lebedev is well taken, even though the amount of excessive moisture retained by a soil under such conditions is rather small, as shown by Lebedev's (10) experiments. We also have to remember that in the Lebedev experiments the soil column is physically homogeneous, which would be true for the filled-in type of lysimeters during the first year. It might also be true for the  $A_1$  horizon. And it will not be true even for the filled-in type of lysimeter where two layers of soil are concerned and less so for the new type of lysimeters where each horizon consists of material of variable physical and chemical composition. In such a case the moisture relationships become more complicated as shown by the ingenious experiments of Lebedev (10). Thus the findings of Lebedev could only partly be applied in the analysis of the moisture relationships in a profile column.

To be sure, a number of reports about lysimeter leachings do show a higher percolation through the deeper lysimeters than through the shallow ones. Lebedev (10) cites the Rothamsted lysimeters, but the data presented by Miller (16) on these lysimeters clearly show that for the first 22 years the reverse was true, namely, the 20-inch lysimeter gave more leachings than either the 40- or 60-inch lysimeters. Only after this period did the deeper lysimeters begin, for a number of years, to show a higher percolation. On the other hand the case of the Plots Experiment Station lysimeters, as quoted by Lebedev (10), show distinctly that the deep lysimeters have percolated more leachings than the shallow ones. The same is true for the lysimeters at the New York State Experiment Station (1).

It is of interest to note that the new type of lysimeters at the Moscow Regional Experiment Station, as reported by Gemmerling (5), also show the tendency to give larger amounts of leachings from the deeper horizons. This is, however, attributed by Gemmerling to the horizontal movement of the moisture along the B horizon from the adjoining more elevated landscape. In our own case the lysimeters are located on a level plot and there is no chance for moisture from the adjoining area to enrich the supply of the lysimeter plot.

One of the serious objections raised against the inclosed type of lysimeters—and this applies to the filled-in type as well as to the built-in type, the Rothamsted type—is that the soil column is completely shut off from the surrounding soil. There can be percolation downward, but no diffusion of water vapor either from below or the sides, no capillary movement from below or the adjacent soil. In the new type of lysimeters this difficulty has been partly overcome. There is practically no interference for diffusion of vapor or capillary

movement, since the funnel is surrounded with soil and we may consider this obstruction as that produced by a shallow stone in the soil. There is this possibility: because of the pebbles in the funnel the moisture content of the soil just above will have a slightly higher moisture capacity, as shown by Lebedev (10). This, of course, might slightly alter the percolation. In general, however, this new type of lysimeter offers an excellent opportunity to study the movement of moisture through the soil profile and incidentally the translocation of the soil constituents in true and colloidal solution.

#### SUMMARY

The scope of lysimeter work has been briefly discussed and a critical analysis of the filled-in type of lysimeter presented.

It has been brought out that the lysimeter work at the Cornell, Geneva, Tennessee, and other stations does not represent natural soil conditions, and the findings are of little value in interpreting the reactions that take place in the soil profile.

A new type of lysimeter installed at the New Jersey station has been described. This type of lysimeter permits the study of the percolation and movement of moisture through the soil profile.

Data on the leachings for 2 years have been presented.

Through the A<sub>1</sub> horizon more moisture percolated during the second year notwithstanding the lower precipitation during this year as compared with the first year. The amount of effective precipitation—that which produced leachings—depends on the condition of the colloids in the soil. No correlation was found between the total and effective precipitation.

A higher percentage of the total rainfall percolated through the A<sub>2</sub> horizon during the first year with a high rainfall than during the second year with a lower rainfall. Again the condition of the colloids seem to be responsible for the phenomenon observed.

Along the B horizon the moisture seems to move in a horizontal direction. This may be inferred from the data on the leachings from B and C.

The data seem to indicate that on a level plot only a small fraction of the rainfall is lost to the ground waters. This might not be true in the coming years, for during the last 2 years the departure from the normal precipitation amounted to 10 and more inches.

It has been brought out that the effective precipitation might possibly be correlated with crop production.

A discussion has been presented on the possible abnormalities of the movement of moisture through soil columns of the filled-in type of lysimeter and the new type, in the light of the findings of Lebedev (10).

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## PLATE 1

### VIEWS OF THE LYSIMETER PIT

- FIG. 1. A general view of the location of the lysimeter pit  
 FIG. 2. Interior view of end of pit where entrance is located  
 FIG. 3. Interior view of end opposite to entrance of pit



FIG. 1



FIG. 2



FIG. 3





# SOLUBLE ALUMINUM STUDIES: I. THE CONCENTRATION OF ALUMINUM IN THE DISPLACED SOIL SOLUTION OF NATURALLY ACID SOILS<sup>1</sup>

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The toxic aluminum theory of soil acidity is based on the assumption that soluble aluminum is found in acid soils in sufficiently high concentrations to be injurious to the growth of plants. Much work has been done in an attempt to prove this theory, as may be seen from the extensive reviews of the literature made by Gile (12), McLean and Gilbert (21), Magistad (22), and others. In spite of the great amount of work that has been done, however, the importance of this theory in explaining the injurious action of acid soils on plant growth is still an unsettled problem. Gile (12) and more recently Line (19) and Hardy (13) have shown the weakness of much of the work upon which the theory is based. Although culture solution studies (21) have well established the fact that aluminum, if present in sufficiently high concentrations, is toxic to plant growth, there is insufficient evidence available from which to conclude that the minimum concentrations found toxic in culture solutions are often found in acid soils. Accurate information, therefore, concerning the concentration of soluble aluminum in acid soils and of factors affecting this concentration is one of the first essentials in a proper evaluation of the toxic aluminum theory of soil acidity.

Three different procedures have been used in studying the soluble aluminum content of soils. The early workers (1, 3, 14, 23) studied the water extract of soils for their soluble aluminum content. This did not prove very satisfactory, however, partly because of the difficulty at that time of determining the small concentrations of aluminum present. The second method of attack involved the use of various salt and acid extracts (4, 7, 9, 18, 20). Although of some value, these methods are open to the serious objection that they do not give the amount of aluminum soluble in the soil water.

More recently a third procedure has been used whereby the displaced soil solution is obtained and is studied for its soluble aluminum content. Since plant roots are in intimate contact with this solution and obtain their nutrients from it, obviously this third general procedure is to be greatly preferred to those

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previously used. Moreover, the recent development of a more accurate method for the determination of small amounts of aluminum makes this general method of attack even more useful.

Burgess and Pember (8) displaced the soil solution of soils from some of the fertilizer plots at the Rhode Island Agricultural Experiment Station and obtained as high as 40 p.p.m. of aluminum in the solution of the untreated soil having a pH of 4.5. Other plots of higher pH values had considerably less or no aluminum in solution.

Magistad (22) and one of the writers (26) have also studied the aluminum in the displaced solution of various soils. The latter found considerably lower values than were found by Burgess and Pember. Moreover, the data of these investigators indicate that different acid soils of similar pH values may contain quite different concentrations of aluminum in their displaced solutions. In view of these facts and the fact that relatively few of the soils used by these investigators were naturally acid soils, it seemed desirable to make a more detailed study of the problem.

The objectives of this investigation are as follows:

To determine the aluminum in the displaced soil solution of a large number of naturally acid soils obtained from various states.

To study the effect of various anions and of total salt concentration on the concentration of the aluminum found in different soils at similar pH values.

To study the seasonal variations in the aluminum concentration of the soil solution.

To study the effect of leaching soils on the concentration of aluminum in the displaced solution.

#### METHODS OF PROCEDURE

Bulk samples of naturally acid field soils were obtained from nine states.<sup>3</sup> A description of these soils is given in table 1. All the samples except soil 533, 537, and 538 were collected in the early spring of 1931. In most cases they were allowed to dry either before or after shipping and the displacement was made after rewetting the soils to the proper moisture content and after allowing them to stand three or four days for the establishment of equilibrium. Soil 554 and the last six soils of table 1 were obtained from the field at approximately the optimum moisture content and were displaced either on the same day or on the following day. This was also true for the soils given in table 3. Other soils used in this study will be described later.

The soil solutions were obtained by the displacement method of Parker (23) as modified by Burd and Martin (5). Successive portions of the displaced solution were tested for conductivity and for pH in order to make sure that there was no leaching effect in obtaining the solution. In many soils the first 50-cc.

<sup>3</sup> The authors wish to express their appreciation to the following men for sending samples of soils from their respective states: Prof. S. D. Conner, Indiana; Dr. J. A. Chucks, Maine; Dr. R. P. Thomas, Maryland; Dr. A. H. Meyer, Louisiana; Dr. D. R. Dodd and Dr. E. E. Barnes, Ohio; Dr. T. E. Odland, Rhode Island; Dr. N. A. Pettinger, Virginia; and Dr. J. A. Bizzell, New York,

TABLE 1  
*Description of soils*

SOIL NUMBER	SOIL TYPE	STATE	SOIL PROVINCE	FERTILIZER TREATMENT*	VEGETATION	PLANT GROWTH
533	Wheeling fine sandy loam	W. Va.	River Flood Plains	Light	General crops	Poor
537	Newton fine sandy loam	Ind.	Glacial Lake	None	Natural prairie	Poor
538	Clermont silt loam	Ind.	Glacial and Loessial	None	Timothy	Poor
544	Caribou loam	Maine	Glacial and Loessial	Heavy	Potatoes	Good
546	Elkton silt loam	Md.	Coastal Plains	None	Native grasses and shrubs	Poor
547	Keyport loam	Md.	Coastal Plains	None	Native grasses and shrubs	Poor
548	Portsmouth loam	Md.	Coastal Plains	None	Native grasses and shrubs	Poor
551	Leonardtown silt loam	Md.	Coastal Plains	None	General crops	Poor
554	Dekalb loam	W. Va.	Appalachian	None	Native grass	Poor
561	Monongahela fine sandy loam	W. Va.	River Flood Plains	Light	Pasture	Poor
562	Peat	La.	Coastal Plains	.....	.....	.....
567	Canfield silt loam	Ohio	Glacial and Loessial	Medium	General crops	Good
569	Brooke silt loam	W. Va.	Limestone	None	Meadow	Fair
570	Miami silt loam	R. I.	Glacial and Loessial	Heavy	Shrubs	Fair
571	Miami silt loam	R. I.	Glacial and Loessial	Heavy	Vegetables	Poor
572	Miami silt loam	R. I.	Glacial and Loessial	None	R. I. Bent Grass	Fair
573	Bladen very fine sandy loam	Va.	Coastal Plains	None	Cut-over timber	Fair
574	Lenore fine sandy loam	Va.	Coastal Plains	None	Cut-over timber	Fair
575	Onslow silt loam	Va.	Coastal Plains	None	Cut-over timber	Fair
576	Dunkirk silty clay loam	Va.	Coastal Plains	None	General crops	Poor
580†	Canfield silt loam	N. Y.	Glacial and Loessial	.....	General crops	Poor
583	Dekalb silt loam	W. Va.	Appalachian	None	Weeds only	Very poor
584	Dekalb silt loam	W. Va.	Appalachian	None	Weeds only	Very poor
586†	Huntington silt loam	W. Va.	River Flood Plains	None	Weeds only	Very poor
587	Holston silt loam	W. Va.	River Flood Plains	None	Native grass	Poor
588	Dekalb silt loam	W. Va.	Appalachian	Medium	Corn	Fair
589	Elk silt loam	W. Va.	River Flood Plains	None	Native grass	Poor
590	Elk fine sandy loam	W. Va.	River Flood Plains	None	Native grass	Poor
591	Westmoreland silt loam	W. Va.	River Flood Plains	None	Native grass	Fair
592	Dekalb silt loam	W. Va.	Appalachian	None	Native grass	Poor

\* Refers to general fertilizer treatment during last 10 years.

† Soils 580 and 586 are poorly drained.

portion going through had a slightly higher pH value than succeeding portions; where the difference was more than one-tenth of a pH this first portion was usually discarded. About 200 cc. of soil solution was usually collected for analysis. Determinations of pH on the soil solution were made by means of the quinhydrone electrode, whereas the determinations on the 1:5 soil-water extract were made by the dialysis-colorimetric method (27). Nitrates were determined in the soil solution by the phenoldisulfonic acid method; chlorides, by titrating with silver nitrate, using potassium chromate as an indicator; sulfates, by the benzidine sulfate method (15); and inorganic phosphates, by the blue colorimetric method as modified by Parker and Fudge (25).

Aluminum was determined by a slight modification of the "aluminum" method described by Winter, Thrun, and Bird (29). Iron and aluminum were first precipitated as the phosphates at pH 4.8 to 5.0. The precipitate was then dissolved in 5 *N* nitric acid and the iron separated from the aluminum by precipitating with 5 *N* NaOH. Centrifuging was used instead of filtering in separating and washing the precipitates. After adding the "aluminon" reagent the solutions were kept at room temperature instead of at 80°C. A series of aluminum standards was prepared at the same time as each set of unknowns, and the colors compared at the end of 20 minutes. With each set of determinations a blank was run on the reagents and corrections were made for it.

#### RESULTS OF INVESTIGATION

##### *Concentration of aluminum and other ions in the displaced soil solution*

The data obtained from a study of the soil solutions of the 30 naturally acid soils from different states are given in table 2. It will first be noted that the pH of the soil extracts varies from 4.0 to 5.0, whereas that of the displaced solutions varies from 3.91 to 4.95. In most cases the soil solution is found to be more acid than the extract, although the difference is unusually slight.

The aluminum concentrations in the displaced solution range from 27.25 p.p.m. for soil 584, which has a pH of 3.91, to a trace for soils 576 and 587, which have pH values of 4.75 and 4.95 respectively. In general, the soils of lower pH contain more aluminum in solution than those of higher pH. However, it is readily evident from an examination of the data that the hydrogen-ion concentration is not the only factor determining the concentration of aluminum in the displaced solution. This is well shown in figure 1. At approximately the same pH values, soils 533, 538, and 571, for example, have from 11.8 to 15.8 p.p.m. of aluminum in solution, whereas soil 572 has only 2.25 p.p.m. Moreover, soils 548 and 573, of considerably higher degree of acidity have only about one-third to one-half as high a concentration of aluminum as soils 533, 538, and 571. Soils 544 and 586 likewise have much higher concentrations of aluminum than other soils of similar or even considerably higher degrees of acidity. These differences in the soluble aluminum concentration of different soils of similar pH values are in agreement with some previous results of one of the writers (26).

The specific resistance of the solutions obtained from the different soils varies considerably. It will be noted from a study of these data that the salt concentration of the solutions, as measured by their specific resistance, influences markedly the concentrations of aluminum at a given pH. The five soils

TABLE 2

*Aluminum concentration in the displaced soil solution as affected by H-ion concentration and the concentration of various anions*

SOIL NUMBER	SOIL MOISTURE AT DISPLACE- MENT (DRY BASIS)	H-ION CONCENTRATION		Al	SPECIFIC RESISTANCE (25°C.)	NO <sub>3</sub>	Cl	SO <sub>4</sub>	INOR- GANIC PO <sub>4</sub>
		Extract	Soil solution						
	per cent	pH	pH	p.p.m.	ohms	p.p.m.	p.p.m.	p.p.m.	p.p.m.
533	12.8	4.58	4.40	15.80	243	200.0	315	77	N. D.*
537	16.2	4.70	4.60	0.50	593	36.0	200	152	Trace
538	17.4	4.45	4.45	11.80	190	57.8	1,320	20	N. D.
544	24.0	4.95	4.87	1.96	281	122.5	335	868	0.48
546	....	4.80	4.60	0.54	2,584	6.8	58	Trace	0.22
547	13.7	4.70	4.80	1.03	2,281	2.0	82	25	N. D.
548	20.9	4.23	4.25	3.88	1,459	Trace	54	238	N. D.
551	23.2	5.00	4.70	2.38	972	61.3	87	15	N. D.
554	17.0	4.53	4.60	3.05	1,901	21.5	25	81	Trace
561	11.6	4.83	4.60	1.31	1,122	57.5	70	Trace	0.22
562	63.0	4.28	4.21	1.00	457	192.3	60	180	0.60
567	19.7	4.40	4.16	5.75	495	212.5	66	Trace	Trace
569	16.4	4.50	4.34	5.50	261	34.7	1,045	149	0.16
570	17.7	5.00	4.70	1.69	548	56.3	223	149	Trace
571	19.6	4.50	4.45	12.25	148	387.5	216	2,067	0.48
572	15.5	4.53	4.43	2.25	1,133	38.8	128	Trace	0.20
573	22.4	4.35	4.07	5.15	1,579	2.2	126	Trace	0.88
574	....	4.35	4.04	11.82	974	6.2	222	Trace	0.20
575	18.8	4.25	4.00	1.50	399	28.0	495	115	0.09
576	19.8	4.88	4.75	Trace	1,168	46.0	120	Trace	0.35
580	25.2	4.03	4.05	11.63	844	100.2	52	29	0.20
583	22.2	4.00	3.98	22.63	862	14.9	168	155	0.14
584	20.6	4.00	3.91	27.25	898	13.9	149	154	0.28
586	27.8	4.20	4.00	27.20	486	40.0	58	894	Trace
587	19.8	4.85	4.95	Trace	2,268	9.7	90	Trace	0.19
588	17.1	4.60	4.52	3.25	486	50.3	396	21	0.34
589	....	4.95	4.94	0.41	1,032	15.1	206	Trace	Trace
590	19.6	4.60	4.51	0.56	3,429	2.7	48	Trace	0.18
591	26.2	4.68	4.64	0.40	1,714	2.7	126	Trace	0.20
592	24.0	4.45	4.55	0.88	2,209	Trace	37	Trace	0.50

\* N. D. means not determined.

previously discussed which were found to have relatively high concentrations of aluminum at their respective pH values are found to be very low in specific resistance or very high in salt concentration. This is readily seen from figure 1. On the other hand, the solutions from soils 546, 547, 587, 590, and 592 which

The effect of soluble salts on the concentration of aluminum at any pH value is further illustrated by a comparison of soils 570, 571, and 572. These soils were obtained from the fertilizer plots of the Rhode Island Agricultural Experiment Station (8). Sample 570 was taken from plot 23, which has received a complete fertilizer, including ammonium sulfate plus lime; sample 571, from plot 82, which has received muriate of potash and superphosphate; and sample 572, from the check or untreated plot of the same experiment. It will be noted that samples 571 and 572 have approximately the same pH. The high concentration of salts, however, which had accumulated in sample 571 as a result of poor plant growth and of continued fertilization and consequent

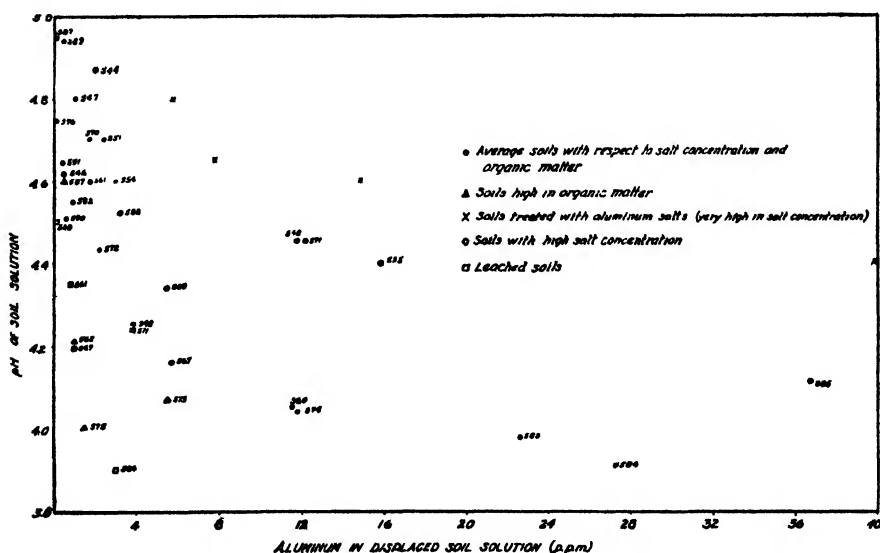


FIG. 1. EFFECT OF pH ON CONCENTRATION OF ALUMINUM IN DISPLACED SOIL SOLUTION

As is to be expected, a low specific resistance is associated with a high concentration of the various anions. With reference to the five soils previously mentioned which have high concentrations of salts, sulfates are especially high in 544, 571, and 586, whereas chlorides are the most abundant anions in 538 and 533. Nitrates are also abundant, especially in soils 533 and 571. This indicates that all three of these anions are instrumental in bringing aluminum into solution or in determining the concentration of aluminum present at any pH value. The phosphate concentration in the soil solution is very low, and it does not seem to have much influence in determining the amount of aluminum in solution. As will be seen later, however, large amounts of phosphate may

reduce the solubility of aluminum. Therefore, with soils which receive large amounts of phosphate fertilizer and possibly those which have relatively low concentrations of the stronger anions, the phosphate anion may have a marked effect in reducing the concentration of aluminum in the soil solution.

Three soils, 537, 562, and 575, have particularly low concentrations of aluminum when their pH values and salt concentrations are considered. It is significant, however, that all three of these soils are very high in organic matter. This is especially true of soil 562, which is a peat from Louisiana. The only other soil in this group of relatively high organic matter content is soil 573.

It should be noted that soil 554 and the last six soils of table 2, which were displaced immediately after removal from the field, have, in general, a relatively low salt concentration and a low concentration of aluminum as compared with other soils of similar pH. The only exception is soil 588, which, unlike the other six, was under cultivation. This brings out two points that should be emphasized. In the first place, some of the soils which could not be displaced immediately after removal from the field but which were allowed to dry and later moistened for displacement are probably higher in salt concentration and in their content of soluble aluminum than they were when removed from the field. This would be especially true of soils 533 and 537, which had been kept in the greenhouse for some time before displacement. Secondly, since it is known that the hydrogen-ion concentration and soluble salt content of soils may vary with cropping and with the season, it may be expected that the concentration of aluminum present in field soils may vary considerably from time to time.

#### *Seasonal variation in the aluminum concentration of the soil solution*

In order to study the effect of season on the concentration of aluminum found in field soils, samples of six of the soils described in table 1 were taken at different times during the year. All of these soils except one were in native vegetation or in grass. Samples taken from the grass areas consisted of at least five shovelfuls of soil taken from an area of less than 10 feet square, each successive sample being taken from within 1 foot of the former sample. In all cases except one, the soil solutions were obtained on the same day that the soils were taken from the field, or else on the following day.

The results obtained are given in table 3. It will be noted that samples taken from the same area at different times during the year vary considerably in aluminum concentration. Moreover, it is evident that this variation is closely related to changes in pH and to changes in the salt concentration of the displaced solution. Soil 554 had about  $2\frac{1}{2}$  times as much aluminum in solution during the middle of the summer, when its hydrogen-ion and salt concentrations were highest, as it did in January or in the following October.

Sample BA of soil 554 was similar to sample B except that it was allowed to remain at optimum moisture content in the soil sample room for nearly two months before being displaced. As a result of bacterial action and a lack of



leaching there were a decided increase in soluble salts and in acidity, and consequently, a nearly threefold increase in the aluminum concentration of the soil solution.

In table 4 are given additional data on the seasonal variation in the aluminum concentration of three small plots of the same soil which had been acidified

TABLE 3

*Aluminum concentration in the soil solution of naturally acid field soils at different times during the year*

SOIL		DATE OF SAMPLING	SAMPLE NUMBER	H-ION CONCENTRATION			Al IN SOIL SOLUTION	SPECIFIC RESISTANCE (25°C)	NO <sub>3</sub> NITROGEN IN SOIL SOLUTION	PO <sub>4</sub> IN SOIL SOLUTION
Number	Type			H <sub>2</sub> O	Soil extract	Soil solution				
				per cent	pH	pH	p.p.m.	ohms	p.p.m.	p.p.m.
554	Dekalb loam	January	A	17.0	4.53	4.60	3.05	1,901	21.5	N. D.
		May 21	B	16.5	4.35	4.39	4.75	2,209	22.4	0.06
		July 10	BA*	. . .	. . .	4.15	12.00	794	115.0	Trace
		July 10	C	14.8	4.40	4.15	7.75	891	38.0	Trace
		October 17	D	15.2	4.60	4.47	3.20	1,872	Trace	N. D.
587	Holston silt loam	May 21	A	19.8	4.85	4.95	0	2,268	9.7	0.15
		July 10	B	22.8	4.40	4.40	0.88	3,741	Trace	0.11
		October 17	C	22.3	4.93	4.77	0	2,072	None	N. D.
588	Dekalb silt loam	May 21	A	17.1	4.60	4.52	3.25	486	50.3	0.34
		July 10	B	15.5	4.93	4.60	1.88	367	176.0	0.34
		October 17	C	15.4	5.03	5.05	0	1,253	40.4	N. D.
590	Elk fine sandy loam	May 21	A	19.6	4.60	4.51	0.56	3,429	2.7	0.18
		July 10	B	18.1	4.60	4.45	1.25	1,198	31.0	Trace
		October 17	C	17.0	4.75	4.66	0	4,324	None	N. D.
591	Westmoreland silt loam	May 21	A	26.2	4.68	4.64	0.40	1,714	2.7	0.20
		July 10	B	21.0	4.60	4.35	1.22	880	Trace	0.07
592	Dekalb silt loam	May 21	A	24.0	4.45	4.45	0.88	2,209	Trace	0.50
		July 10	B	23.2	4.85	N. D.	0.28	648	Trace	0.07
		October 17	C	26.8	4.90	4.86	0	2,026	Trace	N. D.

\* Sample BA is the same as B except that it was kept moist for nearly 2 months before displacement.

with sulfuric acid. Since the sulfuric acid had been added nearly 3 years previously and since two crops had previously been removed from these plots, the soils were probably at much the same kind of equilibrium as if they had become acid naturally. The data further illustrate the fact that changes in pH and in salt concentration which may result from cropping, leaching, and from seasonal

variation in the biological activities in the soils (28), influence very markedly the soluble aluminum content at different times during the year.

*Effect of leaching on the concentration of soluble aluminum in soils*

The effect of leaching on the soluble aluminum content of soil solutions was studied with five soils used in the former studies. These soils vary in the aluminum concentration of their solutions from 1.31 to 27.25 p.p.m. Fourteen kilograms of each soil were placed in pots and leached with 16 liters of distilled water. The soils were then allowed to dry for about a week, at the end of which time they had reached a moisture content optimum for displacing the solution.

The data on the analyses of the solutions are given in table 5. It will be seen that leaching the soils reduced very markedly the aluminum concentration of the soil solutions. The greatest changes occurred with soil 569, where the

TABLE 4

*Aluminum concentration in the displaced solution of artificially acidified soils at different dates*

PLOT	DATE OF SAMPLING 1931	CROPPING DATA	H-ION CONCENTRATION		SPECIFIC RESISTANCE (25°C.)	Al	NO <sub>3</sub>
			Soil extract	Soil solution			
			pH	pH	ohms	p.p.m.	p.p.m.
Barley A	April 7	Barley seeded	4 25	4.40	425	2 00	56
	June 15	April 10, harvested	4 20	4 00	625	2.63	133
	July 6	July 4, 1931	4 25	4 05	489	5.25	115
Rye A	June 29	Rye seeded October 2, 1930; harvested June 24, 1931	4 20	4 00	400	9.25	15
	October 3		4 40	4 20	298	14 70	109
Rye B	June 29		4 58	4 45	681	0 58	38
	October 3		4 60	4 35	256	4 50	80

reduction was from 5.50 p.p.m. to a trace and with soil 584, where the concentration of 27.25 p.p.m. was reduced to 3.00 p.p.m. Although in two cases this decrease might be partly explained by a slight decrease in acidity, the main reason is evidently the marked decrease in the salt concentration as is shown by the data on specific resistance and by those on the concentration of the various anions. The slight increase in salt concentration as measured by the specific resistance and in chlorides in soil 561 may probably be explained by the fact that the soils were allowed to remain moist for several weeks between the time of the first displacement and leaching. The concentrations of total salts and of aluminum just prior to leaching were, therefore, probably higher than is shown in the table.

*Effect of different anions in bringing aluminum into solution*

Since the investigations of Joffe and McLean (16, 17) show that aluminum is soluble at higher pH values as the chloride or nitrate than as the sulfate, it

seemed of interest to compare the effect of additions of aluminum sulfate, aluminum nitrate, aluminum chloride, and phosphoric acid on the concentra-

TABLE 5  
*Effect of leaching soils on the aluminum concentration in the displaced solution*

SOIL NUMBER	LEACHING TREATMENT	H-ION CONCENTRATION		Al	SPECIFIC RESISTANCE (25°C.)	NO <sub>3</sub>	Cl	SO <sub>4</sub>	PO <sub>4</sub>
		Soil extract	Soil solution						
		pH	pH	p.p.m.	ohms	p.p.m.	p.p.m.	p.p.m.	p.p.m.
561	None	4.83	4.60	1.31	1,122	58	70	Trace	0.22
	Leached	4.97	4.35	0.81	1,010	31	156	Trace	0.06
567	None	4.40	4.16	5.75	495	213	66	Trace	Trace
	Leached	4.65	4.20	0.88	1,134	65	64	Trace	Trace
569	None	4.50	4.34	5.50	261	35	1,045	149	0.16
	Leached	5.02	4.50	Trace	1,344	16	112	Trace	0.15
571	None	4.50	4.45	12.25	148	388	216	2,067	0.48
	Leached	4.37	4.25	3.83	671	39	64	344	0.17
584	None	4.00	3.91	27.25	898	14	149	154	0.28
	Leached	4.25	3.90	3.00	1,768	12	76	Trace	0.06

TABLE 6  
*The concentration of aluminum and other ions in the displaced solution of a soil treated with various aluminum salts or with phosphoric acid*

POT NUMBER	TREATMENTS		H-ION CONCENTRATION		Al	SPECIFIC RESISTANCE (25°C.)	NO <sub>3</sub>	Cl	SO <sub>4</sub>
	Kind of solution	Amount of solution*	Extract	Soil solution					
		cc.	pH	pH	p.p.m.	ohms	p.p.m.	p.p.m.	p.p.m.
1-2	None	...	5.40	5.80	0.5	280	320	126	180
3-4	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	120	4.53	4.60	14.9	207	240	93	1,989
5-6	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	224	4.30	4.35	58.5	199	104	66	2,808
7-8	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	344	4.05	4.20	272.0	108	400	261	4,113
9-10	Al(NO <sub>3</sub> ) <sub>3</sub>	120	4.70	4.80	5.8	143	850	118	246
11-12	Al(NO <sub>3</sub> ) <sub>3</sub>	224	4.43	4.40	40.2	98	1,350	68	Trace
13-14	Al(NO <sub>3</sub> ) <sub>3</sub>	344	4.00	4.00	665.0	47	3,150	118	Trace
15-16	AlCl <sub>3</sub>	120	4.63	4.65	7.8	103	330	2,420	137
17-18	AlCl <sub>3</sub>	224	4.38	4.35	63.5	72	150	4,740	106
19-20	AlCl <sub>3</sub>	344	4.00	4.20	296.4	54	120	6,685	120
21-22	H <sub>3</sub> PO <sub>4</sub>	240	4.78	4.90	1.2	191	440	175	442
23-24	H <sub>3</sub> PO <sub>4</sub>	688	4.68	4.60	0.4	285	280	102	397

\* Refers to cc. of N solutions per 8,000 gm. of soil.

tion of aluminum in the displaced soil solution. Three different amounts of each of these salts and of phosphoric acid were added to duplicate pots of a

Wheeling fine sandy loam. These pots were kept at optimum moisture content at the greenhouse for about 4 months before their displaced soil solution was obtained. The analyses of the solutions are given in table 6. As was to be expected, the additions of the aluminum salts decidedly increased the acidity of the soil. The increase in acidity alone, however, does not explain the extremely high concentrations of soluble aluminum obtained. It will be readily noted from figure 1 that the concentration of aluminum in the soils treated with the different aluminum salts is much higher at given pH values than for normal acid field soils. The high values are no doubt primarily due to the high salt concentration which resulted from the addition of aluminum salts and possibly also from bacterial action during the incubation period.

No differences in the direct effect of the different anions, chlorides, nitrates, and sulfates on the aluminum concentration were obtained. This is best seen by plotting the pH values of the solution against their concentration of aluminum. Some of the points are shown in figure 1, and it will be noted that they form a rather smooth curve. Apparently the differences in soluble aluminum obtained from the addition of equivalent amounts of these salts are due to slight differences in the pH of the solutions. This substantiates some of the results obtained from the study of the miscellaneous soils, namely, that high concentrations of any one of these three anions in the soil result in a high concentration of soluble aluminum in the displaced solution. This would seem to be in contradiction to the work of Joffe and McLean (16), who found that aluminum sulfate was less soluble in water solutions at certain pH values than aluminum chloride and nitrate. It must be remembered, however, that these investigators worked with pure solutions. It is possible that under other conditions in soils, as might be obtained at higher pH values or when only one kind of anion is present, differences might be found in the aluminum concentration at similar pH values, dependent on whether the anions are sulfates, nitrates, or chlorides.

On the other hand it will be noted from table 6 that the addition of large amounts of phosphoric acid increased the hydrogen-ion concentration of the soil without greatly changing the aluminum in solution. This is what would be expected since aluminum phosphate is readily precipitated except at very low pH values (13, 19).

*Statistical analysis of aluminum content, hydrogen-ion concentration, and specific resistance<sup>4</sup>*

It is obvious from the data presented that some interrelation exists among the aluminum content, hydrogen-ion concentration, and specific resistance of the displaced soil solution. In order to determine more precisely the magnitude of this interrelation, correlation studies were made of the data presented in table 2. For this statistical study soils 537, 562, 573, and 575 were omitted

<sup>4</sup> The statistical analysis was suggested by Dr. R. J. Garber, head of the department of agronomy and genetics.

because, as has already been explained, they are high in organic matter and are not typical mineral soils. In all calculations the acidity of the soil solution was expressed as hydrogen-ion concentration rather than pH.

The simple linear correlation between aluminum content and specific resistance was found to be  $-0.422$ , after making the usual correction for the small number making up the sample ( $n = 26$ ). This correlation is not high but it has some significance. According to Fisher's tables (11) the  $P$  value is slightly less than  $0.05$ . The chance of obtaining as good or better correlation from uncorrelated data is therefore approximately 1 to 19. With a correlation coefficient ( $r$ ) of  $-0.422$  the coefficient of determination ( $r^2$ ) becomes  $0.178$ . In terms of aluminum content and specific resistance this means that approximately 18 per cent of the variance in aluminum content is associated with that in the specific resistance of the displaced soil solution.

In a similar manner, the linear correlation between aluminum and hydrogen-ion concentration was found to be  $+0.584$ , which is highly significant and indicates a marked relationship. The coefficient of determination ( $r^2$ ) in this case is  $0.729$ . In other words, approximately 73 per cent of the variance in aluminum content may be accounted for by the variance in hydrogen-ion concentration.

Since simple linear methods show a significant correlation in both of the relationships it seemed desirable to use multiple correlation methods to determine the combined effects of the two independent variables (specific resistance and hydrogen-ion concentration) on the dependent variable (aluminum). These give a coefficient of total correlation ( $R$ ) of  $0.877$ . The corresponding coefficient of total determination is therefore  $0.770$ , showing that 77 per cent of the variance in aluminum concentration is explained by the combined linear effects of hydrogen-ion concentration and specific resistance. This value is slightly higher than the simple correlation between aluminum and hydrogen-ion concentration. It is apparent that by taking into consideration specific resistance, 14.9 per cent of the variance in aluminum concentration left unexplained by hydrogen-ion concentration has been accounted for. This means that there is a partial correlation coefficient between aluminum and specific resistance of  $0.386$ . The partial correlation coefficient between aluminum and hydrogen-ion concentration is  $0.849$ .

While making these linear analyses certain graphs were prepared which gave an indication that the relationships could best be explained by curvilinear correlation. The short-cut method as given by Ezekiel (10) was used for this analysis. When applied to the data this method gives a corrected  $P$  value (index of total correlation) of  $0.939$  and a  $P^2$  (index of total determination) of  $0.881$ , which shows that about 88 per cent of the variance in aluminum can be explained by a curvilinear relationship with hydrogen-ion concentration and specific resistance. This value as compared to  $0.770$  for coefficient of total determination shows that the variance explainable by the use of curvilinear relationships is considerably greater than that by the use of linear relationships.

The analyses show very clearly that the aluminum content of the displaced soil solution is very closely associated with hydrogen-ion concentration and specific resistance and that these two variables are of primary importance in explaining variations in the aluminum present in the soil solution.

#### GENERAL DISCUSSION

The data presented in this paper establish the fact that there is not just one solubility curve for aluminum in the soil solution at different pH values. This is contrary to the conclusions of Magistad (22). This author in a very comprehensive investigation of the subject of the toxic aluminum theory determined the solubility of aluminum sulfate in water at different pH values, and also the solubility of aluminum in displaced soil solutions of different reactions. He concluded that "since the two curves practically coincide, one can predict with a fair degree of certainty the amount of soluble aluminum present in a soil solution on knowing the pH of the soil." The explanation of Magistad's results probably lies in the fact that he obtained most of his data from soils treated with different amounts of acid. The soil solutions were, therefore, probably all high in total salt concentration. In the present investigation it has been shown that the salt concentration of the displaced soil solutions materially affects their concentration of aluminum.

The indication that organic soils or soils high in organic matter have low concentrations of aluminum in solution even when very acid is in accord with the observations of Burgess and Pember (8), that "large amounts of decomposing organic matter are efficient in counteracting the deleterious effects of active aluminum upon sensitive crops." It may also help to explain why good crops are sometimes grown on very acid peat soils containing plenty of calcium (2) since these soils are probably also low in soluble aluminum.

Another factor which may possibly affect the concentration of aluminum in the soil solution at any pH value is the percentage base saturation of soils. Other things being equal, soils of relatively high percentage base saturation at low pH values might be expected to have relatively less aluminum in solution than others of low percentage base saturation. Some indication of this was found in a previous investigation (26).

The concentration of aluminum in the displaced soil solution was found to vary greatly from time to time during the season. Not only was this found to be associated with changes in pH but also with changes in the soluble salt content of the soils. Any factor that influences either or both of these properties of an acid soil will, therefore, influence the concentration of soluble aluminum present. Among such factors may be listed the following: presence or absence of a crop, kind of crop grown, biological activities in the soil, weather conditions as they may affect biological activities and leaching of soluble salts, drainage, addition of fertilizers, moisture content of soils.

Examples of the operations of these factors can readily be found in the data presented. Relatively high concentrations of aluminum were found associated

with: (a) the absence of a crop, and the action of biological agents, such as in samples "Rye A" and "Rye B" of table 4, and in soils 533 and 538; (b) the absence of good drainage, as in soils 580 and 586; (c) and the addition of soluble fertilizer salts, as in soil 571. On the other hand, relatively low concentrations of aluminum were found associated in these acid soils with the presence of a growing crop, as in the last four soils of table 2 and in the "Barley A" soil of table 5, and with leaching, as shown by the results given in table 4.

That soils vary considerably in the salt concentration of their displaced solution has also been shown by Burd and Martin (6). They not only found seasonal variations in the soil solution, but they showed that there is an inverse relation between the concentration of total salts—and of certain very soluble ions such as chlorides and nitrates—in the soil solution and the moisture content of the soil. Therefore, in a period of little rainfall, the amount of moisture in the soil may be so reduced as to increase very materially the soluble salt content and thereby the concentration of aluminum in the soil solution. On the other hand, heavy rains accompanied by leaching will decrease the soluble aluminum concentration.

In considering the data presented, it must be remembered that these soils are very acid. Nevertheless, large areas of such soils are found in the northern humid sections of the United States. They represent many different soil types and with one exception are naturally acid soils. A few of the soils which had a very high concentration of aluminum, however, are not truly representative of the average acid soil under field conditions, for they contained a very high concentration of salts.

It is evident from these data that it is difficult to assign definite values for the amount of aluminum in soil solutions at certain pH values. In general, however, it may be concluded that the displaced solution of soils of pH 4.5 or above probably rarely contains under field conditions more than 5 p.p.m. of aluminum, soils of pH 4.8 seldom more than 2 p.p.m., and soils of pH 5.0 seldom more than 1 p.p.m. of aluminum. In the presence of a crop and where frequent rains keep the soluble salt content of the soil very low, the concentration of aluminum in the displaced soil solution may be less than 1 p.p.m. at pH 4.5 and zero at pH 4.8 or above.

These data are believed to be important in determining the validity of the toxic aluminum theory of soil acidity. Not only do they help to explain certain conflicting results regarding the soluble aluminum concentration of acid soils, but they should help in interpreting some of the results of culture solution work and in determining the importance of soluble aluminum as a factor in explaining poor plant growth on acid soils. A more detailed consideration of these relationships will be given in some of the subsequent papers of this series.

#### SUMMARY

Thirty very acid soils representing some principal soil types were obtained from nine states and the relations between their soluble aluminum content and

the concentration of hydrogen and other ions and salts in the displaced soil solution, were studied. The results show that:

The concentrations of aluminum in different soils of similar pH values vary greatly. For example; at pH 4.0, it ranges from 1.5 to about 23 p.p.m.; at pH 4.5, from 0 to about 12 p.p.m.; and at pH 4.9, from 0 to about 2 p.p.m.

The concentration of soluble salts in the soil affects very materially the concentration of aluminum in solution at any given pH value.

The presence of high concentrations of the anions, chlorides, sulfates, and nitrates seem to be equally effective in maintaining a high concentration of aluminum in the soil solution.

Soils having high contents of organic matter contain much less aluminum in solution at given pH values than soils low in organic matter.

A statistical analysis of the data by linear methods showed that 73 per cent of the variance in the aluminum concentration of the soil solution can be accounted for by its relationship to hydrogen-ion concentration. Of the remaining variance, approximately 15 per cent was explained by variation in the specific resistance of the solutions. By using curvilinear methods, it was found that 88 per cent of the variance in aluminum was associated with variations in hydrogen-ion concentration and specific resistance of the soil solutions.

A number of soils in native grass and in general crops were studied at different times of the season for their soluble aluminum content. There was found a marked seasonal variation in the aluminum concentration of the soil solution, and this variation was found to be associated with variations in the soluble salt content.

Leaching soils high in soluble aluminum was found to decrease very greatly the aluminum concentration in the soil solution.

Addition of different amounts of aluminum salts to soil was found to result in a very high concentration of aluminum in the displaced solution.

Attention is called to the various factors which may influence the seasonal variations in the pH and total salt content of soils and consequently the aluminum concentration in the soil solution.

It is emphasized from a consideration of these data that there is not just one solubility curve for the concentration of aluminum in displaced soil solutions at various reactions.

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# MULL AND DUFF<sup>1</sup> AS BIOTIC EQUILIBRIA

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The father of modern forest soil science, P. E. Müller, looked upon the humus layer in the forest as a biological unit. He regarded the strikingly different forms which it assumes in nature as immediately caused by corresponding differences in the visible or invisible "microcosm" composing the living part of the humus layer. The more recent developments in the field seem to indicate a distinct change in attitude, a trend leading away from Müller's biological viewpoint, approaching in many respects the chemical conception favored by the Liebig period. Today the biological characteristics are mostly regarded as merely indicative of the different types, as conditioned rather than conditioning (53, p. 525-526). The responsible factors are sought largely in chemical variations, either primary or secondary (climatic), of the mineral soil or of the organic materials that are undergoing decomposition.

Two things, in particular, seem to account for the decreasing weight given to Müller's biological ideas and for the emphasis again being placed on chemical considerations. One is the attitude of the leading German authority, Ramann. Although he was aware of the importance of biological factors, Ramann does not seem to have been in sympathy with Müller's unitary outlook. He preferred to study single aspects rather than the biological complex as a whole, just as in classification he chose to deal with the isolated components of the humus layer rather than with the humus layer itself (52, pp. 569, 573). It is probably due to Ramann's influence that even Müller's fundamental notion of the *humus layer* has not been much used outside of Scandinavia. Another, and probably the most important, factor which has contributed to the disregard of Müller's ideas is the overwhelming influence of the brilliantly developing regional soil science. As a result, the biological factors, including the higher vegetation, have come to be regarded more or less as merely tools of climate in determining or modifying soil conditions (52, p. 569-570).

The modern views characterized in the foregoing are shared even by workers who have approached the field as biologists. For instance, Hesselman considers the influence of the vegetation as an indirect effect of the climate (19, p. 205, 516). He explains the rôle of the tree species in influencing the type of humus layer on the basis of chemical differences of the

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<sup>1</sup> The terms employed in the present paper ("humus layer," "mull," "duff," etc.) have been defined previously (52). "Duff" has been retained as a term to cover P. E. Müller's "Mor" type of humus layer, although this proposal has met with some opposition on the ground that the word has been used and is being used in other senses. The writer, although feeling this criticism to be perfectly justified, has chosen to retain the term for the present, because all his efforts have failed to obtain an agreement on some other term. If "duff" cannot finally be agreed on by a sufficiently representative majority, the writer would propose for consideration the word "sweard," (the old Anglo-saxon form of the modern "sward"). This root, common to the teutonic languages, and meaning a pelt or hide either on a pig, etc., or on the ground, would seem particularly fit to cover the notion, and in the form "sweard" it would at the present time be absolutely distinctive.

<sup>2</sup> The laboratory work involved in the present study, and much of the field work in the four type localities, has been done by Mr. G. Cavetz. Some of the sampling in the field (soil and plants) was done by Mr. C. Heimburger. To both, the author's thanks are due.

litter. He regards the different development of the humus layer, starting from the same primary material, as essentially due to a different rate of decomposition which is partly governed by and partly governing the balance between the supply of bases and their loss due to leaching (19, p. 357-365, 531-538).

In the present paper, some facts (partly new, but mostly collected from the literature) will be brought together which seem to indicate that the chemical viewpoint current today, useful as it is, is somewhat onesided. Moreover, it will be shown that a number of empirical data can be more easily explained biologically and from the general standpoint held by P. E. Müller.

Parts 2 and 3 of the paper will appear in a later issue. In part 2, an effort will be made to indicate the biological differences between humus layers of different type and to suggest their significance. In part 3, the silviculturally important question of the effects of various disturbances will be treated from what is believed to be somewhat new points of view.

## I. TYPE VERSUS RATE OF DECOMPOSITION

### *Historical*

In P. E. Müller's "Studies," a different *rate* of decomposition is barely mentioned (38, p. 70; 40, p. 57) in his discussions of the differences between the mull and the duff type of humus layer. It is true that Müller has several statements regarding an apparently extremely slow decomposition in the duff, but these refer very specifically to certain brownish fungous mycelia, and not to the duff as a whole, nor to its decomposing plant residue in general. Only in one place (38, p. 35; 40, p. 30) is the statement made that this "almost indestructible" fungous mycelium "imparts some of its resistance to the duff." Instead, Müller lays the stress on the different mode or *type* of decomposition (38, p. 8, 17, 43, 45, 68, 70, 72, 81; 39, p. 78, 147, 155, 159-160, 162; 40, p. 8-9, 15, 36, 37, 55, 56, 58, 65, 178, 234, 240, 243, 245). This attitude is based on extensive and careful observation, as is usual in Müller's work. It is not accounted for by the fact that he shared the common, mistaken (50) view of a poor aeration being generally characteristic of the duff. In fact, his lack of emphasis on the rate of decomposition, as a distinctive difference between mull and duff, is in a way in peculiar contrast to his conception of duff formation as a more or less anaerobic process, altogether parallel to peat formation (he even called the duff "Torf" in the German edition of his studies).

Ramann, the leading authority in the later development, from the first stressed the rate of decomposition as a fundamental difference between mull and duff. The best forest soils, he said, are poor in humus, because of the rapid decay (46, p. 349-350; 47, p. 153; 48, p. 169). He ascribed duff formation to a slow decomposition (46, p. 177-179, 198, 201, 220; 47, p. 92-93, 131; 48, p. 463, 467). Yet, in the first edition of his textbook, Ramann assumed a difference between mull and duff, also in the type of the decomposing processes. A duff results, he said, "when conditions are unfavorable to decay and putrefaction predominates" (46, p. 231). In the following editions, the last three words have been cancelled (47, p. 159; 48, p. 193). Ramann evidently favored the idea of differences in the mode of decomposition only as long as he believed that they corresponded to Liebig's distinction between decay and putrefaction.

When he abandoned this view (48, p. 148), a different rate of decay remained as the essential difference between mull and duff formation.

Today the rate of decomposition is so generally regarded as a fundamental difference between mull and duff, as well as between better and poorer duff conditions, that it seems unnecessary to verify this by references. Statements in case can be found in almost any recent work touching upon the subject of humus forms. This is true whether or not the author in question still believes in a general poor aeration in the duff type. It is sufficient to refer to some of the most recent textbooks and summaries (8, p. 188; 17, p. 321-323; 43; 54; 65; 53, p. 524). P. E. Müller himself, in his later writings, apparently stressed the rate of decomposition more than he did in his early works (41, p. 208, 211; 42, p. 93).

### *Significance of the humus content*

The curious thing is that the universal belief in the rapid decay in the mull and the comparatively slow decomposition in the duff is scarcely based on actual tests of any sort. It is true that both the direct observations in the field and the determinations of humus content seem to indicate so definitely a difference in the direction postulated that it may seem unnecessary to make an effort to prove it. The good crumb mull is usually covered with a loose layer of litter hardly more than 1 year old. It has a low humus content, often (especially in Europe) as low as 10 per cent or lower. The duff includes still recognizable remains from many years of litter-fall, and has a humus content often amounting to 80 or 90 per cent or even more. Thus a duff layer of 1 dm. thickness might seem to represent an enormous accumulation of humus as compared to a mull layer of the same thickness, and the common belief is that it does. It seems somehow to have been forgotten that the low figures for the humus content of the crumb mull are due largely to the fact that they are currently expressed on a weight basis. For a systematic comparison of the humus accumulation, the figures should, of course, be expressed on the basis of volume, or on a unit area of soil down to a certain depth.

Two things have probably tended to distract the attention from the humus content of the crumb mull, and to focus it somewhat one-sidedly on the unincorporated humus. One is the fact that outside of Scandinavia P. E. Müller's fundamental conception of the humus layer has never been commonly adopted. Thus the crumb mull is usually regarded as a part of the mineral soil, and is often left out of consideration in the discussion of humus accumulations. Another probable cause is a logical error which seems to have originated with Ramann (47, p. 141; 48, p. 154). He criticized the idea of Kostytschew that the amount of humus present was in inverse measure to the rapidity of decomposition, inasmuch as the humus must tend to accumulate until a balance is reached between the annual supply of organic matter and its decomposition. Ramann could not see how any regime of that sort could be established, and gave his own opinion in the words: "Accumulation will only take place where the formation of organic materials is more rapid than their destruction. The more this is the case, the greater the increase in quantity of the humus material will be; if both values are only slightly different, there will *seemingly* be a balance, such as has often been assumed." In other words, Ramann looks upon any humus accumulation as initiating a continuous piling up of organic

matter which will never stop as long as conditions remain unchanged. This idea, of course, is very apt to center the attention on the organic material on the surface, much of which is only imperfectly decomposed. This may partially account for the tendency to take for granted that any duff formation, or even the presence of any unincorporated humus, represents an abnormal, progressive accumulation, in contrast to a crumb mull condition, where the balance between litter production and decomposition is supposed to be perfect, or nearly so.

It is easily seen that Ramann's theoretical standpoint is wrong in principle. It would be correct if the forest soil could be compared to a cow and the annual litter-fall to the annual supply of hay reserved for this particular animal. The feeding capacity of the cow is limited, and if the hay supply is greater, some must be left for the next year, and so on. There would be a surplus of hay piling up from year to year. But the decomposition of the litter in nature is not taken care of by any particular animal with a limited food capacity, nor even by a fixed number of organisms. An increased supply means an almost immediate increase in the soil population taking part in the decomposition. With a heavier litter-fall, the total amount decomposed will be greater, and it seems reasonable to assume that, as long as secondary disturbing effects are not involved, it will increase in about the same proportion. In other words, it seems reasonable to consider the characteristics of any given combination of climatic and site factors, as far as decomposition is concerned, as the power of breaking down in a given time a certain *percentage* of the supply of fresh litter, whatever its absolute amount.

The decomposition of any particular batch of litter will proceed at a decreasing rate, as time goes on, following a curve characteristic of the particular litter and of the type of decomposition involved. If the litter-fall is doubled, there will be twice as much to decompose, and twice as much actually decomposed, the first year. There will also be twice as much left for the same or other organisms to work on, the next year, and they in turn will do twice the work, until complete decomposition occurs. If a level of doubled annual litter-fall is maintained, material of different age-classes will pile up until the combined annual loss (which is mainly due to decomposition) corresponds to the annual gain in the form of litter. When this regime is established, the actual amount of material present will be exactly doubled, compared to the earlier conditions with half the amount of annual litter-fall.<sup>3</sup> On the other hand, if the rate of decomposition could be altered without changing the characteristic curve representing the course of the decomposition, such a change would have the same effect as increasing or decreasing the amount of annual litter-fall. Halving the rate of decomposition would mean simply that everything which took one year before will take two years now. When the new regime is established, there will be twice as much material present as before, if the litter-fall has remained the same.

Kostytschew's idea is consequently right in principle. With a given litter-fall, the amount of organic matter present on and in the soil under balanced conditions ought to be a certain measure of the rate of decomposition characteristic of the particular environment. It must be stressed, however, that it cannot be a very accurate measure, not even under strictly virgin conditions. It is hardly imaginable that two different environments will be characterized by widely different *rates* of decomposition without the *type* of decomposition also being different and consequently the characteristic decomposition curve.

<sup>3</sup> This reasoning requires, of course, that the earlier conditions had lasted long enough, so there was another regime established before the doubling of the litter-fall. It is also required that all other conditions, except the amount of litter-fall, have remained unchanged. The latter condition, it is true, will hardly ever apply *exactly* to an actual case; no doubt an incipient humus accumulation, to whatever cause it is due, will as a rule imply or entail a certain change in the conditions governing the decomposition.

Closer comparisons will be permissible only between humus layers of approximately the same type.

Although this reservation has to be made, it is of interest to compare the amount of organic matter accumulation, even with different *types* of humus layer. If the current view is correct, that the essential differences are in *rate*, not in *type* of decomposition, such comparisons are perfectly legitimate. Therefore, if a comparison should fail to show a significant difference in humus accumulation between mull and duff, under comparable conditions, this would be enough to dispose of the ideas favored today.

### *Amount of humus in mull and duff profiles*

Thus far, no systematic comparison of the main types of humus layer seems to have been made with respect to the humus content by volume or area. Only very few of the numerous humus determinations made have been recorded on such bases. Not even all of these figures can be directly used in a critical comparison, because data are lacking on thickness or type of the humus layer, or on the depth to which the determination was extended.

Some very instructive data have been collected by Vater and his collaborators (64). The thickness of the humus layer was measured in place (numerous measurements on each sampling spot were averaged), and on carefully cut-out samples of  $\frac{1}{4}$  sq. m. size the dry weight and loss on ignition were determined. The data permit a computation of the amount of organic matter to the liter of the humus layer in place. Living roots were removed and thus are not included in the weight. All determinations refer to pine and spruce duff. The following figures, computed from Vater's data (64, p. 152, 170-173), show the organic matter content in kilograms to the liter.

SPRUCE DUFF		PINE DUFF	
Upper ("Moder")	Lower ("Torf")	Upper ("Moder")	Lower ("Torf")
0.06	0.07	....	0.08
.. .	0.14	....	0.15
0.09	0.24	....	0.09
0.12	0.15	0.10	0.16

The figures do not in general include the litter, which of course as a rule is still lighter (two determinations for the "Streu" layer on pine duff gave 0.04 and 0.10 kgm. to the liter). Only the first figure of upper spruce duff (0.06) includes some litter, which could not be removed from the F-layer. It is seen that Vater's figures for duff mostly lie near 0.1 and that only one exceeds 0.2 kgm. to the liter even for the lower and more compacted layer.

In crumb mulls, humus contents of 7 to 10 per cent by weight are not rare, even in Europe. In northeastern United States values above 10 per cent seem to be more common than those below 10 (52, table II, p. 589). The dry volume weight of a crumb mull usually does not differ very much from unity [the usual range is .8-1.0 (6, 55)]. Thus, the per-cent-by-weight figures can, for a rough comparison, be directly translated into kilograms to the liter by dividing by 100. The figures obtained are of the same order as Vater's, 0.1 kgm. to the liter. This means that the ill-fated German "*Trockentorf*" does not necessarily contain more organic matter to the liter than a good crumb mull. A 10-cm. layer of "*Trockentorf*," which is commonly considered as representing a very strong humus accumulation, may very well be matched in this respect by a good crumb mull.

Krauss and Grosskopf (22) determined the amount of organic matter on the surface of the soil in a number of German forests with "Auflagehumus" evidently including pronounced duff types. Their figures are expressed in weight per unit area and include the litter, the unincorporated humus, and also the washed-in soil humus, when this was considered necessary to get a true picture of the surface accumulation. The values in general lie between 5 and 15 kgm. to the square meter, although on moist sites in northwest Germany (Erdmannshausen; formerly Neubruchhausen) there are values as high as 40 kgm. to the square meter (estimated from the graphical tables, assuming that those lacking a reference scale are drawn to the same scale as the rest; this is the only way of using the data as they are presented). Except for these extreme values, the figures fail to indicate any stronger humus accumulation than can be found in good crumb mulls.

Auten (2) has some determinations of the weight of duff samples 1 foot square from different forest types on the Mont Alto State Forest in Pennsylvania. Rejecting his figure 1030, table 4, p. 28, which does not check with the data given on weight and thickness, and averaging the remaining 10 determinations, one obtains a weight of 652 gm. for a volume of 144 cubic inches. This is a volume weight of 0.276. Unfortunately, Auten does not give the loss on ignition figures for these particular samples, and there is a great variation in this respect in his material. Using his average figure, 55.5 per cent (2, p. 47), one obtains a humus content of 0.15 kgm. to the liter. This value, uncertain as it is, gives an interesting comparison with Vater's determinations.

A few additional data on volume weight and humus content of American duff forms were obtained in the writer's laboratory on samples taken by Mr. C. Heimburger in the Adirondack Mountains. The samples were taken using steel cylinders of Burger's (6) model (10 cm. high and of 1 liter capacity). Localities with particularly thick duff were chosen. One was a moss-rich red spruce flat, with fibrous duff, between Polliwog Pond and Middle Pond. The other was a hemlock ridge with a heavy greasy duff near Floodwood railroad station. Both were in the Saranac region. Only the H-layers were sampled. The figures obtained are as follows:

	FIBROUS DUFF	GREASY DUFF	
		Upper H	Lower H
Dry volume weight. . . . .	0 14;0 145	0 18	0 245
Ignition loss, <i>kgm. to the liter</i> . . . . .	0.12;0 12	0 12	0 14

These figures evidently corroborate the conclusions drawn in the foregoing from Vater's data, inasmuch as the values check very well with his. Even for the very compact-looking greasy duff the humus contents do not much exceed 0.1 kgm. to the liter. It should be noted that in these determinations *living roots were not picked out*, contrary to the procedure of Vater and of Krauss and Grosskopf. The values for the root-free humus would consequently have been still lower, no doubt especially so for the fibrous duff. Vater in one case (64, p. 170) gives the weight of the roots picked out. They represented 16 per cent of the total fresh weight of the humus layer.

The figures hitherto presented pertain exclusively to the humus layer. It is evident, however, that in order to judge the amount of humus accumulation the organic content of underlying strata should also be taken into consideration. When the available figures are examined, it is at once clear that here great variations are to be expected. The following general considerations seem, however, to be warranted.

A crumb mull is in many regions typically combined with a "brown soil" profile (48, p. 533; 61). The humus content of the brown horizon often amounts to 3 per cent or more in

the upper parts of the layer (60, p. 129, 149; 23, p. 18). It decreases very gradually with increasing depth. A characteristic of a typically developed brown forest soil is that the colloid-rich horizon is very deep [often up to 70 cm. (61, p. 5)].

A pronounced duff, on the other hand, is typically associated with a podzol profile. The various types of podzol profiles are widely different. In some of them, the humus content of the accumulation horizon may rise to values not encountered in any brown forest soil. In others, the humus content may be low throughout, even in the horizon of accumulation. The leached layer, too, sometimes contains very considerable amounts of humus, whereas in other cases the quantity is small. If the particularly humus-rich types of podzol profiles, associated with a more or less swampy condition, are excluded, there remains among the rest a widely distributed type of forest podzol profile: the iron podzol of Frosterus. This is typical of large stretches of coniferous forest with fibrous duff on normally drained soil (59, p. 55; 62). Frosterus (16) states as one of its characteristics that the humus content of the illuvial horizon is below 3 per cent (in another place: usually below 3). Now in this type the illuvial horizon is normally richer in humus than any other horizon of the mineral profile. Furthermore, the horizon usually fades out rapidly with increased depth (59, p. 58), so the well-developed part of it certainly averages less than the brown layer of typical brown forest soils. All this does not indicate in general any greater accumulation of humus in the mineral soil under fibrous duff than in the brown forest soil under mull.

More significant than such general considerations are direct numerical comparisons of duff and mull profiles, preferably in the same region. Unfortunately, very few figures are available of total humus content, expressed on the basis of volume or area. Consequently resort must be made to the usual per-cent-by-weight data, reasonable volume weight values being assigned to the different layers of the profile. In the computations, the following volume weights have been used throughout: 0.8 for crumb mull, 1.0 for the upper and 1.2 for the lower brown soil; and 1.5 for all mineral horizons of podzol profiles, as well as for the subsoil in all profiles. The figures are chosen with a view of being certain that the humus content of the brown forest soil profile with mull is not calculated unduly high as compared to the podzols with duff. For the same reason, the depth of 1 m., to which the computation has been extended, has been counted from the top of the mull in mull profiles, but from the top of the mineral soil in duff profiles.

A comparison has been made on this basis between three type profiles from Sweden chosen and carefully described by Tamm. The first is a very typical brown forest soil profile with mull from Hissón, province of Småland, south Sweden (23, p. 16-18; 57, p. 168). The humus content to the square meter was estimated as follows:

11 cm. crumb mull = 110 l. = 88 kgm. with 9.7 per cent humus, makes	8.5 kgm.
17 cm. upper brown soil = 170 l. or kgm. with 4 per cent humus, makes	6.8 kgm.
28 cm. lower brown soil = 280 l. = 340 kgm. with about 2 per cent humus, makes	6.8 kgm.
44 cm. subsoil = 440 l. = 660 kgm. with about 1 per cent humus, makes	6.6 kgm.
Total humus content to the square meter down to 1 m. depth	28.7 kgm.

To this figure should be added the organic matter of a litter cover 2-3 cm. in depth.

For comparison, two duff profiles from northern Sweden have been chosen, representing different shades of the iron podzol as developed under the influence of spruce forest of the *Myrtillus* type, the strongest duff-forming and strongest podzolizing among all the forest types of north Sweden occurring on normal, not swampy, ground (59, pp. 154, 159, 166). The first locality, representing, it seems, the purest "*Vaccinium*" type of Malmström (34, 35), is near Håsjö, province of Jämtland, near the geographical center of Sweden (59, pp. 249-



250; 62, pp. 325-327; 23, p. 34). Assuming for the humus content of the (fibrous) duff the rather liberal figure 0.15 kgm. to the liter (cf. the foregoing), the estimate runs:

4 cm. duff = 40 l., estimated humus content 0.15 kgm. to the liter, makes	6.0 kgm.
8 cm. leached layer = 80 l. = 120 kgm. with 2.2 per cent humus, makes	2.6 kgm.
10 cm. rust-brown layer = 100 l. = 150 kgm. with 3 per cent humus, makes	4.5 kgm.
82 cm. subsoil = 820 l. = 1,230 kgm. with 1.2 per cent humus, makes	14.8 kgm.
Total humus content to the square meter down to 1 m. depth,	27.9 kgm.

To the figure should be added the organic matter of a litter cover 1-2 cm. thick.

The second duff locality seems to represent a somewhat moister variant of Malmström's "*Vaccinium*" type (with *Dryopteris Linnaeana* and *Cornus suecica*) from Rokliden, province of Norrbotten, north Sweden (59, pp. 246-247; 62, p. 327; 23, p. 35-36; 57, p. 168). The analyzed profile was exceptional in having a very heavy, hard ortstein, the upper 15 cm. of which were dark brown. The estimate works out:

7.5 cm. duff = 75 l., estimated humus content .15 kgm. to the liter, makes	11.3 kgm.
20 cm. leached layer = 200 l. = 300 kgm. with 1.4 per cent humus, makes	4.2 kgm.
15 cm. upper ortstein = 150 l. = 225 kgm. with 2.5 per cent humus, makes	5.6 kgm.
30 cm. lower ortstein = 300 l. = 450 kgm. with 1.7 per cent humus, makes	7.6 kgm.
35 cm. subsoil = 350 l. = 525 kgm. with .6 per cent humus, makes	3.2 kgm.
Total humus content per square meter down to 1 m. depth,	31.9 kgm.

To the figure should be added the organic matter of a litter cover 2-3 cm. thick.

Considering the approximate nature of the estimates, the three values obtained might well be regarded as identical. In the first locality, the mull was, in places, 15 cm. thick, which would make the figure of the total humus content 31 kgm. to the square meter instead of 29. Conversely, if in the estimate for the last locality the more conservative and probably better value .12 is used instead of .15 for the humus content of a liter of duff, the total humus content would become 30 kgm. to the square meter instead of 32. The result of the comparison should then be expressed thus: *A type profile of brown forest soil with crumb mull from south Swedish beech-woods shows as high a total humus content as either of two type profiles with pronounced duff from the strongest duff-forming and podzolizing forest type on normally drained ground in northern Sweden.*

Hardly anyone would have expected this result from an ocular comparison of the two profiles from Hissön and Rokliden.<sup>4</sup> It also controverts absolutely ideas current in the literature. The writer recalls only one statement of recent date which allows any possibility of such a result. This statement is found in P. E. Müller's latest work (42, p. 96). In discussing the amount of nitrogen contained in heath soil he says that this depends on the amount of humus, "which in heath areas with a thick duff cover *in general* exceeds that of the mull *on the same sandy soil.*" (The italics are mine.) In order fully to realize the remarkable cautiousness of this statement it must be remembered that the typical profile of the Danish heaths with its heavy, compact duff, its strongly humus-impregnated, partly black and greasy leached layer, and its partly black ortstein (66, plate I) gives every indication of representing a particularly strong humus accumulation. It should also be remembered that the mull in the oak scrubs representing the mull islands in the heath is particularly light in color and poor in humus. Nevertheless, P. E. Müller by his "*in general*" grants the possibility of the heather profile being not *always* the richer in humus, not even when it has "a thick duff cover." Furthermore,

<sup>4</sup> Both these places have been visited by the writer.

only the mull "*on the same sandy soil*" is included in the comparison, not mull or crumb mull in general.<sup>5</sup>

The chief reason for the apparently common error of judgment concerning these matters is probably a theoretical conviction that the pronounced duff profiles *must* represent a particularly strong accumulation of humus. Secondly, there is an overrating of the humus content of humus layers of the duff type, because so very few determinations of their volume weight have been made.

The comparisons just made of course do not necessarily show anything about the rate of decomposition in the different types, because there is no guarantee that the annual litter-fall is comparable in the different localities. No determinations seem to have been made of the litter-fall of north Swedish softwoods but there is reason to believe that at least in the northernmost locality it is considerably smaller than in the beech forest in south Sweden where the first profile was located (cf. foot-notes 6 and 11).

Data which would seem to permit a comparison under conditions of about equal litter-fall are reported in a paper by Bujakowsky. He gives (5, p. 263) figures of "total organic matter" in kilogram to the square meter for a number of localities in German spruce forests (of different history, it is true; some following oak, some pine, some spruce, etc.). The data, however, do not represent the total organic matter down to a standard depth. Only the strongly humus-impregnated surface layer has been taken into account, down to a depth varying from 5 to 40 cm. (in most cases 5-10 cm.), counted from the top of the "mineral soil" (personal communication from Dr. Bujakowsky). For instance, in the most pronounced crumb mull profile (No 1) only a 5- or 6-cm. surface layer has been considered, out of "20 cm. loam rich in mull, well crumbled." It seems evident that Bujakowsky's "totals" are grossly misleading, particularly for the mull profiles; they can hardly be used for a critical comparison.

Some very significant figures have been recorded recently by Morgan and Lunt (37). They compared the total amounts of organic matter down to a depth of 40 inches in two pairs of profiles of different type, situated two and two in the same neighborhood (one pair in the White Mountains and one in Connecticut). Translated into kilograms per square meter and given in condensed form, their figures are:

<i>Data given on locality</i>	<i>Total organic matter kgm. per sq. m.</i>
Thick podzol, Waterville, N. H. . . . .	62.1
Rich crumb mull, Bethlehem, N. H. . . . .	57.8
Thin podzol, Union, Conn. . . . .	30.4
Crumb mull, Groton, Conn. . . . .	24.9

Of the four profiles studied by Morgan and Lunt, two represent localities visited by the writer: the Waterville tract and the crumb mull locality at Bethlehem. What makes Morgan and Lunt's figures particularly significant and interesting is that the Waterville tract is believed to represent a virgin forest (red spruce, with some admixture of hardwoods) except for the parts where cuttings have been started recently, after the tract was made a part of the White Mountain National forest. In the places examined by the writer, the humus layer was a typical greasy duff, sometimes very heavy (52, Table X, samples 119-121), in fact, much

<sup>5</sup> In fact, the humus accumulation in the Danish heaths does not seem to be excessive. From data recently reported by Weis (66, p. 31, 45, 49) from a virgin heath with a "rather thick" heather duff and a "very strong" hardpan (including a thick, black humus pan), the total organic matter accumulation down to 1 m. depth can be estimated at about 40 kgm. to the square meter. This figure should be compared with some data reported in the following for mull profiles.

heavier than it could have been in the profile studied by Morgan and Lunt. Which one of the two profiles is more representative of the tract, the writer is unable to tell. At any rate it is very remarkable that Morgan and Lunt's Waterville profile—whether chosen at random or with a view of representing average conditions—has yielded so nearly the same total amount of organic matter per unit area as the crumb mull profile at Bethlehem. The latter locality has been described in detail elsewhere (52, appendix p. 16-18). The crumb mull is perfectly typical morphologically. Only it is unusually deep and rich in humus, judged from European experience. The near identity of the total amount of humus in Morgan and Lunt's two profiles from the White Mountains would certainly be surprising to anybody who has seen the profiles.

The general result of the comparisons made is that a typical crumb mull profile may represent just as high an accumulation of organic matter as does a very pronounced duff profile, even when conditions are fairly comparable. *There is no indication of a consistent difference between the crumb mull and even pronounced forms of duff in the amount of organic matter stored up either in the humus layer or in the entire profile.*

Of course the writer does not intend to deny the possibility of a certain average difference between mull and duff in amount of accumulated organic matter. It is realized that there are duff profiles which represent an accumulation of organic matter hardly matched by any crumb mull profile. As an example, the following profile with 40 cm. greasy duff may be quoted (Hemlock ridge near Floodwood station, Saranac region, Adirondacks, sampled by C. Heimbürger).

	kgm. per sq. m.
4 cm. litter and F layer, estimated at .....	4 0
21 cm. upper H layer, 210 x 0.1225 .....	25.7
16 cm. lower H layer, 150 x 0.144 .....	23 0
4 cm. leached layer, 40 x 0.017 .....	0 7
20 cm. ortstein, 200 x 0.105 .....	21 0
20 cm. transition zone 200 x 0.023 .....	4 6
56 cm. subsoil 560 x 0.0085 .....	4 8
Total organic matter content down to 1 m. depth .....	83.8

Even a humus accumulation of this impressive amount does not necessarily mean, however, that the decomposition is excessively slow. It is true that 84 kgm. to the square meter probably equals 200 years or more of the annual leaf-fall;<sup>6</sup> but to the latter has been added, in the virgin forest, about an equal amount (9, p. 68) of organic matter from logs. The accumulation, therefore, probably corresponds to no more than between 100 and 200 years' total litter

<sup>6</sup> The determinations made in central Europe on different species have mostly given values ranging between 0.3 and 0.5 kgm. organic matter to the square meter, with a surprisingly small variation according to site and species (9, 63), exceptionally as low as 0.2 (7) or as high as 0.6. In Danish beech woods, C. M. Møller found values all ranging between 0.2 and 0.3 (unpublished data read August 1929 at the 18th meeting in Copenhagen of northern naturalists; kindly communicated by Prof. Møller). In Jack and Norway pine stands in northern Minnesota, Alway and Zon (1) found as low an average as 0.2. Auten (2) finds in Pennsylvania 0.3 for the ridge, 0.4 for the slope, and 0.6 for the cove type. Unpublished data kindly furnished by Professor Geo. S. Perry, of Mont Alto, Pa., give the values 0.3 for pitch pine, 0.5 for red pine, and 0.6 for white pine stands in Pennsylvania.

production. On the other hand, it represents probably the total result of accumulation from thousands of years of forest growth, maybe all since the glacial period. The greasy duff will not easily burn at any season of the year.

Still it should not even be contested that in particular cases there may actually be a "practical standstill of decomposition," as duff formation has frequently been interpreted in Germany. Some data reported from northwest Germany seem in fact to indicate a really amazing accumulation during a single rotation (see for instance 21, p. 429).

### *Soil respiration*

The production of carbon dioxide gives a fairly good idea of the total decomposition going on in materials like snuff, composts, manure, or soil. It would seem, therefore, that a determination of the  $\text{CO}_2$ -evolution, or so-called respiration, of the soil in place would furnish an excellent means of comparing the rapidity of decomposition in different types of humus layer or soil. In fact, the various authors engaged in such determinations during the last decade frequently have used their results for interpretations along that line. Lundegårdh expresses the idea very clearly: "As would have been expected, the true mull soils respire most intensively, raw humus soils more weakly" (28, p. 182). Later, he was much surprised to find that duff soils also may have a considerable respiration (33, p. 381). Fehér tries to bring the respiration values in parallel to the results of bacterial counts, although not always with success (14, p. 482). It is easily seen, however, that this reasoning cannot be correct, as far as stable natural conditions are concerned. With slow decomposition, more material will pile up than with more rapid, but when a regime is established, the respiration will be exactly equal in both cases, under otherwise equal conditions, particularly if the litter-fall is the same and if there is no difference in the root respiration. *Determining soil respiration under balanced natural conditions is measuring the annual litter-fall plus the root respiration, but not the rapidity of decomposition.*<sup>7</sup>

Yet the data cannot be ignored. It must be realized that the authoritative statement by Lundegårdh, quoted in the foregoing, is incompatible with the conclusion just presented. It is well known that with the same species and apparently very much the same litter-fall either a good crumb mull or a bad duff might form. The beech, for instance, is at the same time one of the best "soil-improvers" and one of the worst duff-formers. According to Lundegårdh's statement, which is based on data from beech forests, beech mull would show a consistently stronger respiration than beech duff. From the reasoning given in the preceding paragraph, this would not be expected, under balanced conditions.

Table 1 gives a synopsis, aiming at completeness, of the available material from European forest soils in place. (Some determinations from this country are reported in the following section.) The table also includes forest soils worked according to silvicultural practices, and soil of cleared areas. Some of these data will be of use for later reference. Treeless heath-

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<sup>7</sup> It is true that in dealing with artificial conditions, it might be quite different. There the method will in many cases be of value in studying the immediate effect of silvicultural measures on decomposition. Effects of that kind will be discussed in a following part of the paper. Here we are primarily concerned with the differences between natural types of humus layer. It is evident from what has been said that we cannot expect to get much pertinent information on that point from data on soil respiration alone.

TABLE 1  
*European determinations on respiration of forest soils*

REFERENCE	PERIOD	LATITUDE, °N.	STAND	GROUND VEGETATION	HUMUS LAYER	RESPIRATION, GM. CO <sub>2</sub> PER SQ. M. PER HOUR			N	METHOD
						Maximum*	Minimum*	Mean		
51, p. 32	VIII. 24-IX. 4	63	Pine (2 stands)	Moss-rich type	Duff	0 62	0?	0 22; 0 49	10; 12	acc
45, p. 124	V 31-VI 1	60½	Spruce + pine	Oxalis-Myrtillus type	Duff	0 60	0 19	0 47	13	vent
45, p. 125	VII 3-4	60½	Spruce + pine	Oxalis-Myrtillus type	Duff	1 28	0 56	0 84	25	vent
45, p. 126	VIII 24-25	60½	Spruce + pine	Oxalis-Myrtillus type	Duff	1 01	0 60	0 85	25	vent
51, p. 32	VII. 28-VIII. 30	59	Pine (2 stands)	Moss-rich type	Duff	1 11	0 32	0 54; 0 54	38; 51	acc
10, p. 328†	VIII. 28-IX 11	56½	Pine	Grass, moss, Myrtillus, etc.	Duff	0 45	0 22	0 30	45?	acc
24, p. 68	VIII. 31	56½	Beech	None	Mull†	0 38	0 35	....	2	sim
50, p. 145	VI. 29-VII. 2	56½	Beech	Scarce; mixed type	Duff	0 25	0 14	0 18	4	acc
10, p. 326†	VII. 14-VIII 3	56½	Beech	Scarce; mixed type	D 6-12 cm.	1 2	0 5	0 87	63?	acc
25, p. 352	X 10	56½	Mixed hardwood	Rubus idæus, Oxalis	Mull†	0 08	....	....	1	acc
24, p. 68	VIII. 11-21	56½	Alder	Oxalis	?	0 25	0 20	0 23	3	sim
24, p. 68	VIII. 12; VIII 21	56½	Alder	Circea; Swamp vegetation	Muck?	0 21	0 17	....	1; 1	sim
25, p. 352	X. 10	56½	Alder	Oxalis, Aspidium; Viola palustris	Mull†	0 12	0 07	—; 0 07	1; 2	acc
25, p. 352	X. 10	56½	Alder	Oenanthe aquatica	Muck	0 03	....	....	1	acc
25, p. 352	X 10	56½	Alder	Maianthemum bifolium	Duff†	0 03	....	....	1	acc
26, p. 137	?	56½	Alder	Not given ("swamp")	Muck?	0 12	0 03	....	2	?
27, p. 24	VIII 22	56½	Alder	Oxalis	Mull†	2 34	....	....	1	acc
10, p. 324†	VII 1-6	56½	Alder	Mixed, mostly swampy	Muck	0 36	0 15	0 24	18	acc
36, p. 39	VIII. 10-30	53½	Spruce + pine	None	5 cm. Li + F	0 83	0 43	0 57	19	sim
36, p. 40	IV. 17-V. 7	53½	Spruce + pine; spruce	None, or Polytrichum	4-8 cm. Li + F	0 45	0 15	0 27; 0 18	21; 6	sim

36, p. 39	VIII. 12-24	53 $\frac{1}{4}$	Spruce + pine	— (soil recently worked)	.....	0 70 0.48	0.60	5	sim
36, p. 40	IV. 18-24	53 $\frac{1}{4}$	Spruce + pine	— (same next spring)	.....	0 18 0.13	0.15	3	sim
36, p. 39	VIII. 10-31	53 $\frac{1}{4}$	Pine stands	Mosses, or no vegetation	Partly D	0 58 0.30	0.39, 0.57	10; 3	sim
36, p. 40	IV. 17-V. 1	53 $\frac{1}{4}$	Pine	Mosses, or no vegetation	Partly D	0.26 0.14	0.19	15	sim
36, p. 72	VIII. 22-IX. 9	53	Pine stands	Mostly grass (some worked)	Sod	0 93 0.25	0.48	29	sim
36, p. 72	IX. 3-9	53	Clear cuts	Mostly grass, mostly worked	.....	0.73 0.21	0.44	10	sim
36, p. 72	IX. 5; IX. 8	53	Clear cut	<i>Just thoroughly worked</i>	.....	1 35 1.17	....	2	sim
36, p. 50	VII. 4-24	52 $\frac{3}{4}$	Pine + hardwood	Duff plants	Duff	0.99 0.39	0.62	46	sim
36, p. 50	VII. 8; VII. 11	52 $\frac{3}{4}$	Pine + hardwood	<i>Duff recently turned over</i>	Duff	1 40 1.13	....	2	sim
36, p. 50	VII. 6-24	52 $\frac{3}{4}$	1 Beech; 2 Oak	Polytrichum, or no vegetation	Mostly D	0.70 0.53	0.60	6	sim
36, p. 63	VIII. 4-18	52	Pine	Moss, Cladina, or none	Mostly thin	0.65 0.30	0.45	34	sim
36, p. 113	VII. 19	52	Pine	Moss, Cladina, or none	Mostly thin	0.50 0.18	0.33	9	acc
36, p. 63	VIII. 5; VIII. 7	52	Beech under pine	Some Polytrichum	8 cm. FD	0.59 0.57	....	2	sim
36, p. 161-170	VI. 2-XI. 24	51 $\frac{1}{4}$	Spruce	None, or mosses, etc.; some Rubus	4-10 cm. D	0.54 0.07	0.31	25	acc
36, p. 163-166	VI. 2-XI. 10	51 $\frac{1}{4}$	Beech; oak + beech	Few grasses, moss, Myrtilus	Up to 10 cm. D	0.84 0.10	0.36	21	acc
36, p. 166-169	VI. 6-XI. 15	51 $\frac{1}{4}$	Beech; oak + beech	None, or Oxalis and some moss	Mull	0.72 0.13	0.33	35	acc
36, p. 170	X. 2; X. 17	51 $\frac{1}{4}$	Pine	Myrtilus, Leucobryum	7 cm. D	0.30 0.20	....	2	acc

\* The values given as maxima or minima as a rule represent single determinations, but in Fehér's series from Hallands Väderö (= 56 $\frac{1}{2}$ ° lat. N.) they are averages for a day (representing each three determinations) and in the same author's 12-month series from Sopron (= 47 $\frac{1}{2}$ ° lat. N.) they are averages for a month.

† Fehér's values from Hallands Väderö as originally given were 10 times too high, because he had used, without checking, the erroneous formula published by Lundegårdh (27, p. 7; 28, p. 146; 30, p. 420) and reprinted by Stoklasa and Doerell (56, p. 688), for computing the results using the accumulation method. The error was pointed out by Romell (51, p. 56), and in later papers by Fehér the data from Hallands Väderö are quoted with their correct values (except 13, table 4, p. 28, where by mistake the original values still stand). The values given here are the corrected ones.

‡ These are Lundegårdh's indications as to the type of humus layer (28, p. 182).

|| Date and ground vegetation as given by Lundegårdh (30, p. 420, and 28, p. 181).

TABLE 1—*Continued*

REFERENCE	PERIOD	LATITUDE, N.	STAND	GROUND VEGETATION	HUMUS LAYER	RESPIRATION, GM. CO <sub>2</sub> PER SQ. M. PER HOUR			N	METHOD
						Maximum*	Minimum*	Mean		
36, p. 35	VII. 6-26	50½	Spruce	None	Weak duff	0.63	0.36	0.47	12	sim
36, p. 35	VII. 12-25	50½	Spruce + hardwood	Moss, grasses, some herbs	"Well decomposed"	0.72	0.55	0.63	9	sim
15, p. 255	VII. 15-25	47½	Oak	Rich herbaceous vegetations, ferns and moss	Mull	1.4	0.8	1.06	11?	acc
15, p. 257	VII. 26-VIII. 8	47½	Pine + hardwood	Few herbs, Pteris, Polytrichum	Mull?	1.15	0.7	0.88	14?	acc
15, p. 258-259	IX. 1-13	47½	Spruce + hardwood	Hazel, herbs, some Myrtillus	Mull?	0.85	0.2	0.56; 0.56	13; 3?	acc
15, p. 260	IX. 13-16	47½	Spruce + larch	Mull mosses	Mull	0.7	0.5	0.58	4?	acc
12, p. 423	12 months	47½	Spruce	Rich herbaceous vegetation, mull mosses	Mull	1.26	0.07	0.58	360?	acc
14, p. 474	VII. 30-VIII. 14	47	Robinia	Grasses, few herbs	?	1.83	0.03	1.21; 0.56	13; 12	acc
14, p. 477	VIII. 31-IX. 7	46½	Robinia	Anthriscus trichospermus, Equi- setum ramosissimum	?	1.60	0.72	1.18	7	acc
14, p. 477	VIII. 31-IX. 7	46½	Austrian pine	None	Duff	1.39	0.04	0.85	7	acc

The figures in the "Reference" column refer to the bibliography. The Roman numerals under "Period" refer to the months, and the Arabic numerals, to the days of the months indicated. The latitudes north are approximate. The humus layer has been labeled either from direct statements on its type made by the respective authors, or by inference from other data given (description, vegetation). A question mark indicates that the labeling is reasonably safe in spite of somewhat insufficient or contradictory indications. The abbreviations in the "Humus layer" column mean: D = duff, F = F-layer, FD = fibrous duff, Li = litter, M = mull (52). The column "N" gives the number of determinations represented by the preceding data. For the last column, see "Comments on methods." Part of Fehér's values have been taken out from his graphs, and the second decimal might in these cases be wrong. The same applies to all of Porkka's values. In some cases, as is seen, the second decimal is not even given.

land and meadows have been left out. Three values reported by Stoklasa and Doerell have been omitted because of the absence of data on analytical methods used and data as to location.<sup>8</sup>

Before quoting the figures, a few comments on methods will be given. They will be of use in judging some of the data, and also for later reference.

*Comments on methods.*—There are three types of methods, which will be referred to as the “simultaneous absorption,” the “accumulation,” and the “ventilation” methods (abbreviated in table 1 as “sim,” “acc,” and “vent” respectively). All three have in common the fact that a small area of soil is isolated under a bell or box, into which the CO<sub>2</sub> diffuses from below. In the *simultaneous absorption* method (4, 24) the CO<sub>2</sub> is absorbed, as it diffuses from the soil, by an alkaline solution contained in a flat vessel placed under the bell. With the *accumulation* method (50, pp. 143–144), the CO<sub>2</sub> gradually accumulates under the bell, where no absorbing agent is present. The rate of accumulation, which is determined on withdrawn air samples, measures the respiration intensity. In the *ventilation* method [proposed independently, it seems, by three different parties (56, p. 689; 20, 44)], the CO<sub>2</sub> evolved from the soil is carried away by a current of air and conducted to an absorption apparatus.

Meinecke (36, p. 106) compared the simultaneous absorption and the accumulation methods and found about 10 per cent higher results for the former. Romell (51, pp. 40–41) pointed out that this result cannot be generalized, and explained the relatively good check obtained by Meinecke as due to the rather long time (24 hours) that he ran his absorption tests. The simultaneous absorption method will in general give more reliable results with a *long* duration of the experiment (51, p. 41), provided the absorption is satisfactory.

With the accumulation method, the tests should be made as *short* as is possible for analytical reasons. The degree of approximation to the true values with this method depends in principle merely on the accuracy of the methods for measuring the CO<sub>2</sub> accumulation. Thus the most sensitive analytical methods should be used, and Fehér (11) did well in replacing the awkward apparatus recommended for the purpose by Lundegårdh (28, p. 147) by a more reliable one (Lundegårdh’s bell apparatus). On the other hand, with Fehér’s use of this combination, there is the possibility of unexpected errors which might be serious. Fehér takes out for analysis .6 to .8 liters, from a diffusion bell having a cross section of about .07 sq. m. This means that when taking his sample for analysis, he sucks out the air contained in the surface soil down to a depth which, with an air space of 40 per cent, amounts to 2–3 cm. Now the CO<sub>2</sub> content of the soil air, even in the surface soil, frequently counts in tenths of a per cent, not in hundredths, as in the free air. The error introduced in the analysis by this excess CO<sub>2</sub> will depend on conditions, for example, on how much of the soil air will actually

<sup>8</sup> Some reader may miss the data given by Lundegårdh in his several later publications. His original data are, however, all in the table. But most of them are difficult to recognize, from his later publications, because a most curious series of calamities happened to them when Lundegårdh compiled the table on p. 181 in his book of 1924. Numbers 7–13 in this table are evidently quoted from Lundegårdh (25, p. 352) but have become 10 times higher than before. [It is regrettable that Lundegårdh did not compute a “diffusion number” for No. 7, as he did for Nos. 8–9, 11–12, because that would have given him a value higher than what he concludes (28, p. 160) to be the highest possible, and thus would have shown him that something was wrong with the figures.] Numbers 14–16 of the table are expressly quoted from Lundegårdh (24, p. 68) but are still more difficult to recognize, having become about 4.2 times higher than they originally were. A condensed result of these slips has unfortunately been perpetuated not only by Lundegårdh himself (29, p. 361; 30, p. 420; 31, p. 408; 32, p. 269; 33, p. 381), but also by Dengler (8, p. 160), partly by Russell (53, p. 311), and possibly by others. In these condensed tables, there is the further slip that the two last lines of the table (28, p. 181) have been interchanged, so the figures which in 1924 referred to “fucoid beds on the shore” have come to stand for “beech forest.”



reach the opening of the sampling tube and be drawn out into the analyzing apparatus. With perfect stirring during sampling, half of the soil air will be included with the sample. Assuming a  $\text{CO}_2$  content in the soil air of .2 per cent, which is hardly an extreme figure, this would make the value determined about 30 per cent too high in a model case quoted by F    r (11, p. 354).<sup>9</sup>

The ventilation method presents a number of obvious technical difficulties, but seems to be without objection in principle, provided the ventilation is effected by atmospheric air and the arrangement is such that the outside pressure is maintained within the bell. (This seems to apply to Porkka's use of the method.)

In judging the figures contained in table 1, it should be noted first that none of the workers engaged in collecting the data has been concerned with the problem of comparing definite natural types of humus layer. The studies were made with other views in mind. This explains why such a great deal of the material refers to forests planted on old heath-land (Meinecke), plantations on sand plains (F    r), and other artificial conditions with humus layers which are often difficult to classify, at least from the descriptions given. Only the Swedish-Finnish material (the localities between 63   and 56     lat. N.) is chiefly composed of determinations from natural forests. Unfortunately, this material very one-sidedly represents the duff type. The only localities labelled as "mull" are all on the little island of Hallands V    r  , and even those are not representative. The good Swedish material from duff soils could for our purposes hardly be used otherwise than for a comparison with material from localities far away.

The sandy and gravelly soil of the granitic island Hallands V    r   in the climate of the western Swedish sea shore permits a typical mull to form only in places, and hardly under pure beech. In the alder swamps, the mull, if it forms at all, is of the detritus mull type, if the writer recalls correctly. Thus a good crumb mull locality has hardly been included in the studies made in Fennoscandia, except possibly Lundeg    rdh's single determination rather late in the fall in a "mixed hardwood" stand on Hallands V    r  . Moreover, even if Lundeg    rdh's "mull" localities are all accepted as such, they represent very few determinations. Because of the dominating r    le they have played in the literature, however, the data from Hallands V    r   will be discussed separately later.

In the German material, too, the localities clearly representing mull conditions are scarce. Only some of the beech stands in Gahrenberg (51     lat. N.) can with certainty be labelled as good mull localities. Fortunately, the series obtained in these Gahrenberg localities are long and representative, and parallel series were run simultaneously in beech stands with pronounced duff. Thus in this case a significant comparison is possible. *It is seen that neither the extremes nor the averages indicate any significant difference.* In fact, the figures for the stands with mull appear a little lower. At least two of the three mull localities included in these series seem from the description to have a clear crumb mull and brown forest soil profile.

The Hungarian material, contrary to the more northern material, mostly represents mull soils, or at any rate soils without duff (some of the localities may hardly have a forest humus layer at all). In fact, only one duff locality is included (labeled as such by F    r). This one locality is a plantation of Austrian pine at Szeged (46     lat. N.) and only seven determina-

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<sup>9</sup> Possibly F    r's values might be too high also for another reason. The time taken as the duration of the test is given as the time "between the closing and the opening of the bell." This is far from being clear, but if it means the time between putting in the stopper in the neck of the diffusion bell and sucking out the sample, there is a possibility of a certain unintentional and undetermined accumulation of  $\text{CO}_2$  in the bell before the experiment was taken to start. (It seems that the  $\text{CO}_2$  value at the start was determined not on a sample drawn from the bell, but in the open air.)

tions were made there. The results average considerably lower than seven parallel determinations in a near-by locust plantation. This could be taken as an argument in favor of Lundegårdh's contention, but it cannot be considered a very strong one. Not only are the determinations few, but the localities at Szeged represent highly artificial and hardly stabilized conditions (17-43-year old plantations on the puszta), so the values obtained cannot be considered as having much bearing on our problem. It should be noted, further, that even the duff locality yielded a high mean and a high maximum value. Both are higher than the corresponding averages for the rest of the Hungarian material.

There remains the possibility of getting something out of a general comparison between mull and duff averages from table 1, independently of the season and the geographical location. One could possibly hope that the extent of the material would be sufficient to offset the disturbing influence of variations of various kinds. Accepting for a moment this reasoning to be justified, one will be surprised, first, to see that the highest mean values north of Hungary have been encountered not in Germany, but in Finland and Sweden. It is noted that these high northern averages represent clear duff types.<sup>10</sup> For the rest, the mean values mostly lie, rather evenly distributed, between 0.2 and 0.7 gm. of CO<sub>2</sub> to the square meter per hour throughout Sweden, Finland, and Germany. The mean value of the long and representative series from mull soils at Gahrenberg in southern Germany is .33 gm. to the square meter and consequently does not rank particularly high, in spite of the fact that these localities mostly represent site-class II (in one case III) for beech.

The apparent lack of a general downward trend in the values towards the north, within the German and more northern material, is not as surprising as it might seem at first hand. It is true that the litter production is probably greater in some of the German material representing high site-classes than it is in the northern stands.<sup>11</sup> On the other hand, the length of the season favorable for decomposition must influence the values. With a longer growing season, the values per hour or day will average lower, under the same conditions of litter-fall, because the same amount of decomposition is distributed over a longer period. This is probably the explanation of the surprisingly high northern values.

All the more remarkable, then, do the high values from Hungary seem. Here the averages to about 50 per cent lie within the same range (of .2-.7 gm. of CO<sub>2</sub> to the square meter per hour) as in most of the more northern material, but the other 50 per cent are higher, some very much so. This is the case also with the one series from a duff locality. It is true that the

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<sup>10</sup> Porkka does not tell anything about the humus layer in his localities, but it is apparent from the vegetation and the location (*Oxalis-Myrtillus*-type near Helsingfors) that it must be a clear duff type. Fehér fortunately gives data on the thickness of the duff in his beech forest on Hallands Väderö. When he calls a 6-12 cm. thick duff "relatively thin" (10, p. 326), it must be remembered that he believed at that time the hourly soil respiration to average 8.7 gm. of CO<sub>2</sub> to the square meter, high above Lundegårdh's earlier world's record of 2.34 gm. to the square meter (see the following), and that this, according to current views, must mean a particularly rapid decomposition and consequently the absence of any marked surface accumulation of humus. The beech forest where he worked represents only site-class V of Schwappach (51, p. 52).

<sup>11</sup> True enough, the litter-fall has been found to vary surprisingly little with the site-class (63). On the other hand, the percentage of the yearly production of dry matter which goes into litter-forming material has been found remarkably constant (9), and the production of wood, as is well known, varies markedly with the site-class. Evidently the absolute amount of litter-fall and the percentage of the production going into the litter cannot both be constant, independently of site-class. The explanation of the relative constancy of both values is probably that in the more productive stands the leaf-mass is somewhat increased, but not in proportion to the greater production, which is in part due to the assimilating apparatus working more efficiently under more favorable conditions of site.

highest values are from other localities, without duff. Thus the general high level of the Hungarian values could be taken as an argument in favor of a greater  $\text{CO}_2$ -production of mull than of duff soils. This argument cannot be given much weight because of the difficulty of a significant comparison.

There is, furthermore, the possibility that the Hungarian values are too high because of the method used (cf. "Comments on methods"). If the total evolution of carbon dioxide during a year is computed from the very interesting all-year series obtained in a spruce forest at Sopron ( $47\frac{1}{2}^\circ$  lat. N.), one finds that it represents the amazing value of 1.39 kgm. of carbon to the square meter of soil, corresponding to the carbon content of over 3 kgm. of litter, which must be between 5 and 10 times the actual amount of needle-fall. Of course, the ground vegetation might have contributed a considerable share, and we do not know how much of the soil respiration is due to the roots. Still the result of the computation appears very high, too high to look quite reasonable.

The survey of the available determinations of carbon dioxide production by forest soils has given a somewhat disappointing result. In the whole material there is hardly more than one case where a significant comparison of mull and duff soils can be made. This one comparison shows no difference. Lundegårdh's contention that the true mull soils respire more intensely than the duff soils is, therefore, hardly supported by evidence.

Lundegårdh's attitude was in fact determined by data representing, in all, eight single determinations. Out of these, five were made on the same day in October when the hourly respiration was in most cases reduced from tenths to hundredths gram of  $\text{CO}_2$  to the square meter. Of the three remaining determinations, which are said to represent "true mull" soils, two have given the perfectly normal value 0.4 gm. to the square meter which is greatly exceeded by many values for duff, even by averages of long series, obtained (by Fehér) on the same island. There remains the single value 2.34 gm. to the square meter, which is indeed very high, representing at present the world's record. This single value does not inspire confidence. It stands quite isolated among over 900 determinations from  $63^\circ$  down to  $46^\circ$  latitude, and is about 10 times higher than all the other data obtained from the same locality (alder forest on Hallands Väderö) during the same time of the year (July–August).<sup>12</sup>

In conclusion, the respiration determinations made on forest soils have shown a considerable variation, but most of the average values lie between 0.2 and 0.7 gm. of carbon dioxide to the square meter per hour. This holds true all the way from the northern part of Sweden at  $63^\circ$  latitude at least down to southern Germany. Thus the normal range seems to be rather different from what has been taught in textbooks for the last seven or eight years. The *minimum* figures exceeding 1 gm. to the square meter, given by Lundegårdh in a number of publications, seem to correspond approximately to normally occurring *maximum* values. Averages up to 1.3 gm. have been found in Hungary, by the use of a method which possibly gives too high values. Contrary to what has been taught, there is no indication of a consistently more intense respiration of mull soils, even crumb mulls, as compared to duff. The regrettable scarcity of the material available for significant comparisons is hardly the sole reason for this negative result. It is really to be expected, and this whether or not there is any difference in rate of decomposition be-

<sup>12</sup> Cf. footnote 2(†) to table 1. The excellent agreement obtained between the directly determined figure 2.34 and the  $\text{CO}_2$  evolution from soil samples (27, p. 24) is hardly a proof that the value, or even its order of magnitude is correct [cf. van Suchtelen's remarkable findings on the behavior of isolated soil samples (58)].

tween mull and duff. The soil respiration cannot well be a measure of this rate, as it has been supposed to be. Under balanced natural conditions, it is rather a measure of the annual leaf-fall plus the intensity of root respiration.

*A comparison between crumb mull and root duff under Northern hardwoods*

In the preceding discussion, the lack of unquestionably comparable data on humus layers of different type is keenly felt. The determinations reported in the following are given as a modest contribution toward the filling of this gap. The work was planned with the view of finding an explanation of a certain discrepancy between field observation and some nitrification tests, reported in an earlier paper (52). With this in mind, it was decided to choose for a comparison the crumb mull and the root duff types of humus layer. The latter was chosen rather than some other form of duff, partly because of its surprisingly high nitrification during storage in the laboratory, partly because in the region root duff rather than more pronounced forms of duff can easily be found near crumb mull and under relatively comparable conditions. Two pairs of localities were selected for the purpose, all included in earlier studies.

Three of the places of study have been previously described (52, appendix, localities 1: A-B, and 10). The fourth is a woodlot with very typical crumb mull near Camillus, New York (52, table VII, samples 122 and 204 a-b). This was used for comparison with the Baldwinsville locality (No. 10). Unfortunately, the crumb mull locality 1: A at Jacksonville had soon to be abandoned, because the owner would not permit further access to his woodlot. Thus the comparison between the two woods at Jacksonville, which would have been particularly interesting, could not be carried out as planned. Instead, a woodlot with mull near Enfield (52, table VII, sample 209b) was chosen for comparison with the root duff locality 1: B at Jacksonville. Unfortunately, the series of determinations could not be made as long as would have been desirable, particularly not for the Baldwinsville-Camillus set.

As a first approach to the problem, nitrate tests were made in the spring on plants of the ground vegetation from crumb mull and root duff at Jacksonville. Such tests give a certain idea of the nitrate level of the soil in which the plants grow, and have been used for this purpose by Hesselman (18), Raunkiaer (49) and others. The tests were made quantitatively using Blom and Treschow's (3) method of analysis.

For a significant comparison, a nitrate collecting species had to be found growing in both localities. It was believed that some of the violets would fulfil the requirements (52, p. 588, footnote). A closer examination showed the impossibility of finding any violets or other nitratophilous plants on undisturbed root duff under the closed stand (52, appendix, p. 29). An attempt was made, however, to pick the specimens as far away as possible from openings. On May 13, 1931, a supply of *Viola pubescens* was collected in this way from both localities. The entire plant except the subterranean parts was taken. Sterile and flowering specimens were kept apart, making four lots in all, each weighing about 5 gm. when oven-dry.

Analyses of portions taken from the dried, powdered, and thoroughly mixed material showed the following contents of nitrate-N in milligrams per gram of dry sample:

	JACKSONVILLE CRUMB MULL	JACKSONVILLE ROOT DUFF
Flowering violets.....	4	0
Sterile.....	4	0

The result agrees with what can be inferred from direct field observation on the flora, and indicates that the storage experiments do not give a true idea of the nitrate level in the two soils. The storage experiments in this case had shown about the same nitrifying power for the F-layer of the root duff as for the crumb mull, sometimes one, sometimes the other, giving the higher value (52, tables VII and IX).<sup>13</sup>

Next, parallel determinations were made of the nitrate content per liter of soil, of the soil respiration, and of organic matter content.

Soil samples were taken with Burger's (6) steel cylinders of 1 liter capacity, 10 cm. high. Nitrates were determined by the phenoldisulfonic method, applied as described previously (52). Soil respiration was determined by the accumulation method (cf. p. 175), using a much simpler apparatus than the author's previous (51) model. The bell, made of heavy galvanized sheet iron, was low and wide, only 10 cm. high, but with .2 sq. m. cross section. A stirring device was provided with two long, narrow wings which could be moved by a crank from the outside. The crank was slowly turned during the whole experiment. The CO<sub>2</sub> accumulation was determined on air samples of 60 ml. volume sucked out by mercury into sampling tubes of glass (with mercury traps instead of stopcocks). One sample was taken at the beginning, another at the end of the experiment; the CO<sub>2</sub> accumulation was determined by the difference. The analyses were made in the laboratory using either a Pettersson or a Haldane standard gas analysis apparatus. The 60-ml. sample was sufficient for a double analysis.

A somewhat radical innovation in the soil respiration technique was that the rim of the bell was not pressed down into the ground. The bell was just placed on the surface, and it was only made certain that there were no visible openings between the rim and the soil. Any such openings were filled from the outside by heaping up some surface material. It is believed that with the great area of the bell and the consequently better proportion between area and circumference, the error due to CO<sub>2</sub> escaping to the sides is small when the tests are short (they were made to run about 10 minutes). It is true that this assumption has not thus far been proved by actual tests. The procedure described was nevertheless adopted because it was considered essential to use a technique disturbing the natural conditions as little as possible. Because of the sparse ground vegetation in the spots tested, one could even refrain from cutting any plants. The few and small green plants sometimes found inside the area enclosed by the bell were left undisturbed. The tests were all carried out in the early afternoon, between 1 and 3 p.m., except for one test made at 4 p.m.<sup>14</sup> In computing the results, the barometric pressures read in Ithaca and Syracuse have been used, corrected for the difference in elevation.

<sup>13</sup> In table IX, samples 332 are by mistake dated July instead of June. Samples 331 and 332 were taken on the same day, just as were the following two and two on the same dates (345-346, . . . 368-369).

<sup>14</sup> This is mentioned because a daily period in soil respiration has been found. It is true that according to Porkka's (45) results the period does not seem very pronounced in forest soils.

The result of the determinations of nitrate content and of soil respiration are assembled in table 2.

It is seen that the nitrate level in the crumb mulls averages about twice as high as in the root duff soils. Where there are any nitrates at all, the values are, even in the individual cases, higher for the crumb mull, except on the two days July 27 and 29, where, for unknown reasons, there was an inversion, both in Enfield-Jacksonville and Baldwinsville-Camillus. There is hardly anything in the meteorological data to account for this freak.

On the other hand, the values of soil respiration are much the same for crumb mull and root duff. When the variation is considered, there is no difference in the averages. The figures are somewhat low, compared to the majority of those previously recorded (table 1), but their absolute height should be left undiscussed until the technique used has been checked.

TABLE 2  
*Nitrates in top decimeter of soil and soil respiration*

DATE	CONTENT OF NITRATE-N IN MG. PER LITER SOIL		RESPIRATION GM. CO <sub>2</sub> PER SQ. M. PER HOUR		LOCALITIES
	Crumb mull	Root duff	Crumb mull	Root duff	
May 27.....	1.7	1.5	.	.	Jacksonville
July 1.....	2.4	0.3	0.24	0.41	Enfield-Jacksonville
13.....	1.0	0.25	0.24	0.18	Enfield-Jacksonville
20.....	1.8	0.0	...	.	Camillus-Baldwinsville
22.....	0.2	0.0	0.30	0.16	Enfield-Jacksonville
27.....	0.6	1.1	0.37	0.12	Camillus-Baldwinsville
29.....	0.0	0.7	0.14	0.19	Enfield-Jacksonville
August 1.....	0.0	0.0	0.24	0.12	Enfield-Jacksonville
6.....	0.0	0.0	0.24	0.17	Enfield-Jacksonville
12.....	0.0	0.0	.	...	Enfield-Jacksonville
14.....	Trace	0.0	0.30	0.32	Camillus-Baldwinsville
Averages.....	0.7	0.35	0.26	0.21	

The conclusion to be drawn from table 2 is that evidently about the same total decomposition plus root respiration was going on in the crumb mull and the root duff soils, but that at the same time the nitrate level was in general much higher in the mulls. The former fact is to be expected from the general similarity in the character of the stands, which probably produce much the same amount of litter and have about the same amount of roots.<sup>15</sup> The latter fact is in complete agreement with the character of the vegetation and with the nitrate tests on violets, reported in the foregoing. Still the conclusion is

<sup>15</sup> The *distribution* of the roots can have no influence on the respiration values (provided the regime established is not disturbed in making the determination), unless the respiration of deeper roots should be depressed by lack of oxygen or excess of CO<sub>2</sub>. This can not be expected to be common in normally drained soils (50).

not permissible that we here have proof that the two types of humus layer are different not in rate, but merely in type of decomposition (cf. the discussion in the preceding section). In order to obtain eventually such proof, the humus contents of the different soils must be considered.

Determinations of organic matter were made on all the 1-liter samples used for the nitrate tests, and in addition on some liter samples taken at greater depths down to 40 cm. The determinations were made by igniting at 550°C. the samples previously dried in a vacuum oven. The determinations include all organic matter present in the samples, whether living or dead (litter, humus, roots). In Enfield, no samples from greater depths could be taken because of the great number of stones in the soil. The results of the determinations on the first decimeter are, in kilograms per hectoliter, i.e. per square meter down to 10 cm. depth:

	ENFIELD- JACKSONVILLE	CAMILLUS- BALDWINSVILLE	MEAN
			<i>kgm per hl.</i>
Crumb mull.....	10 6 $\pm$ 2 0	10 3 $\pm$ 0.47	10 5 $\pm$ 1 05
Root duff.....	6 7 $\pm$ 0 48	6 5 $\pm$ 1 0	6 6 $\pm$ 0 53

The somewhat surprising result is that *the root duff localities average distinctly lower<sup>16</sup> than those with crumb mull.* The determinations from greater depths did not reverse the picture. The results are, in kilograms per hectoliter:

DEPTH  <i>cm.</i>	CRUMB MULL PROFILE— CAMILLUS	ROOT DUFF PROFILES	
		Baldwinsville	Jacksonville
10-20	8 8	3 8	4 3
20-30	7 2	2 0	4 1
30-40	5 8	2 0	Not determined

This gives the following totals down to 40 cm. depth for the two completely examined profiles at Camillus and Baldwinsville:

Crumb mull profile 32.1 kgm. organic matter per square meter  
Root duff profile 14.3 kgm. organic matter per square meter

These figures seem to furnish conclusive proof that in the cases examined the distinctive difference between the two types of humus layer, the crumb mull and the root duff, is not at all in rate of decomposition. Better expressed, the difference is not in the direction which would be expected from current ideas. Under what can be confidently assumed to be very much the same conditions of litter-fall, the root duff has been found to represent a distinctly *lower* accumulation of organic matter than the crumb mull. If a conclusion as

<sup>16</sup> The difference of the means is  $3.8 \pm 1.17$ . The errors were computed as  $\sqrt{\Sigma(d^2):n(n-1)}$ .

to the rate of decomposition is permitted (cf. the discussion in a preceding section), it would be this: that the decomposition proceeds at a *faster* rate in the root duff than in the crumb mull.

At the same time, as shown in the foregoing, the nitrate level was found about twice as high in the crumb mull as in the root duff. Combined, these data seem to permit only one conclusion: that *the essential difference of crumb mull and root duff with respect to decomposition is in the course it takes, not in its rapidity.*

It might be useful to point out that even when referred to weight of organic matter the nitrate content of the crumb mull averaged higher than the root duff (5.2 as against 3.1 mgm. per kilogram organic matter). It is likewise interesting to note that the soil respiration, when referred to unit weight of organic matter present in the top decimeter of the soil, is twice as high in the root duff as it is in the crumb mull (32 mgm. as against 15 mgm. per kilogram organic matter and per hour). These figures seem to be conclusive.

The foregoing findings seem to furnish for the first time proof of the correctness of P. E. Müller's original view, against modern ideas (cf. the historical introduction) and seem to have rather far-reaching consequences, which will be discussed in the following parts of the paper. For the time being, the result stands alone, but data brought together in a preceding section strongly indicate the possibility of this being simply due to the fact that no such analysis has been attempted as has been made in the case just presented. In a way, of course, it can be said that the case analyzed is extreme. The root duff is a particularly light form of duff. Probably there are not many duff forms representing a considerably more rapid decomposition than the crumb mull. This does not make the present findings less interesting. What is important is the proof furnished against the general interpretation of duff formation as implying a slow decomposition, as compared to the supposedly very rapid breaking down of the organic matter in a good crumb mull.

It might be objected that this interpretation of the results is not independent of where the line is drawn between mull and duff. To a certain extent this is true. If the root duff had been included in the mull group, the conclusion drawn in the preceding paragraph would have had to be worded differently. But it should be pointed out that independently of any subtleties of classification there are striking differences, between the places examined, in litter cover and in development of the humus layer. In the mull localities, the litter is very scarce during most of the year, and never includes leaves older than 1 year. An unincorporated humus is totally lacking. In the root duff localities, the litter and F-layer include "little decomposed" remains of several years of litter-fall, and there is a distinct accumulation of unincorporated humus, strongly matted together by roots. These differences are among those generally prevailing between a good crumb mull and a duff. They are currently taken to imply or to prove a more rapid decomposition in the crumb mull type. The present comparative study has conclusively shown that this inference is not correct. It has been demonstrated that the rate of decomposition can



actually be as rapid as in the good crumb mull, or even more so, also where an unincorporated humus is formed and where several years of litter-fall are present in recognizable form. This important result stands independently of any possible controversies on classification.

With regard to the question of the "sampling effect," from which the experimental part of this study started, the results are not conclusive. The low amount of organic matter in the root duff, in particular of the F-layer, which is the chief carrier of the nitrifying activity, the small number of determinations and the great variation in actual nitrate content and nitrification during storage do not permit any definite conclusion. For this reason, the "sampling effect" is better discussed in a following part of the paper, where the question will be attacked from more general points of view.

#### SUMMARY

P. E. Müller's original view of the two main types of forest humus layer (mull and duff) being different in *type* of decomposition rather than in *rate*, is contrasted to modern ideas which consider the duff formation essentially as a result of a stagnation of the decomposing processes. An analysis of data brought together from the literature seems to show that the latter view is a result of optical and numerical illusions. The humus accumulation in duff layers has been overrated, probably mainly because so few determinations of their volume-weight or their humus content per unit volume have been made. Some such determinations from heavy duffs in the Adirondacks are reported. They agree very well with Vater's results from Germany. The total organic matter content of some European type profiles is computed. It appears that typical brown forest soil profiles with crumb mull may represent just as high a humus accumulation as podzol profiles with a heavy duff. Figures recently reported by Morgan and Lunt from this country lead to the same conclusion. An Adirondack profile with excessively heavy duff is computed and it is shown that not even such a great accumulation necessarily implies any "practical standstill of decomposition," as a heavy duff formation has frequently been interpreted.

The material available on "respiration" determinations on forests soils is critically reviewed, and some widespread errors are corrected. It is shown that soil respiration determinations do not necessarily show anything about the rate of decomposition. Under balanced natural conditions the carbon given off as carbon dioxide (plus what is removed by leaching) will correspond to the carbon contained in the litter-fall plus the amount given off by the roots. This will apply whether the decomposition is rapid or slow. The total organic matter accumulation, on the other hand, must be in inverse measure to the decomposition rate, under balanced and otherwise comparable conditions. Ramann's error of judgment on this point is criticized.

A comparison of two pairs of localities with root duff and crumb mull in the Finger Lakes region of New York State has given conclusive proof of these types of humus layer being distinguished by the course which the decomposition processes take, and not by a simple difference in their rapidity. This conclu-

sion is arrived at by determinations of nitrate level, soil respiration, and amount of organic matter down to 40 cm. depth. The respiration was the same for both types; a result to be expected from the close similarity in the character of the stand. The nitrate level averaged twice as high in the crumb mull as in the root duff, but the amount of organic matter was also much higher in the crumb mull profile, meaning that the rapidity of decomposition at least cannot well be higher than in the root duff. With equal soil respiration and different amounts of organic matter, it is evident that the respiration per unit weight of the latter must be different. In fact, it was twice as high in the root duff as in the crumb mull, whereas the nitrate content remained higher for the crumb mull even if referred to unit weight of organic matter. Thus, the higher nitrate level in the crumb mull cannot be accounted for by a more rapid decomposition. The difference is the other way.

The result seems to dispose of several current ideas. It shows that the presence of "little decomposed" remains of several years of litter-fall and the formation of an unincorporated humus do not necessarily imply a slow decomposition, as compared to a good crumb mull condition.

The conclusions drawn will be discussed further in the next part of the paper, to be published in a later issue.

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## COMPARATIVE RATE OF DECOMPOSITION OF COMPOSTED MANURE AND SPENT MUSHROOM SOIL<sup>1</sup>

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In the study of the decomposition of composted horse manure by the cultivated mushroom, *Psalliota (Agaricus) campestris*, it was found that this organism feeds primarily upon the lignins and the proteins of the composts and only to a less extent upon the hemicelluloses and the cellulose (4). This process of nutrition is just the opposite from that of the common fungi and bacteria living in the soil and in composts. In the composting of stable manure, as well as in the decomposition of plant and animal residues in the soil, the microorganisms, including numerous fungi and bacteria, attack—next to the sugars, starches, and proteins—the hemicelluloses and the cellulose; these are reduced in relative concentration more rapidly than the organic matter as a whole. The lignins are more resistant to attack by the microorganisms of composts and soils, and will, therefore, accumulate. In the decomposing plant residues, low in nitrogen, the proteins will accumulate, since they are continuously synthesized in the form of cell substance of microorganisms. When such a compost of horse manure was used for the growth of the cultivated mushroom, it was found (3) that this growth was accompanied not only by a marked decrease in the lignins and in the proteins of the compost, but also by a marked increase of the water-soluble substances, especially the nitrogenous complexes. It was suggested that the first is due to the feeding of the mushroom upon the lignins and the proteins and the second to the synthesis of extensive mushroom mycelium, which penetrates through the compost and a large part of which is water soluble.

In view of the fact that the resistance to decomposition of the organic nitrogenous complexes in composts and in soil is due to their combination with the lignins of the plant residues, to form resistant complexes (2), one would expect that any organism capable of attacking such complexes, as seems to be the case of the mushroom fungus, would bring about the transformation of the nitrogen into a form in which it would become more readily available to the common fungi and bacteria of the soil. As a corollary of these observations, one would expect that composted manure should decompose in the soil much

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more readily after the mushroom fungus has grown upon it than before, and the nitrogen should be liberated more readily in an available form.

In this connection it may be of interest to call attention to the results obtained by Falck (1), who found that tree residues, which have been decomposed by a fungus attacking primarily the lignins will, after such decomposition, be readily attacked by other organisms, whereas the same residues which were first acted upon by organisms attacking the cellulose and not the lignin will not be decomposed later by the lignin-decomposing fungus; in other words, the growth of lignin-decomposing organisms makes the residues more readily decomposed by the common microbial flora of the soil, whereas the growth of cellulose-decomposing organisms makes the residue more resistant to decomposition.

TABLE 1  
*Chemical composition of fresh and composted horse manure before and after the growth\* of Agaricus campestris*  
Per cent of dry matter

NATURE OF MATERIAL	FRESH MANURE		COMPOSTED MANURE		
	Control	<i>Agaricus</i>	Control	<i>Agaricus</i> I	<i>Agaricus</i> II
Ether-soluble portion . . . . .	2 46	1 08	1 26	0.48	0 43
Water-soluble organic matter . . . . .	6 31	34 22	3 24	18 70	25 05
Hemicelluloses . . . . .	18 10	5 50	6 80	2.64	2 02
Cellulose . . . . .	21 40	9.70	12 51	7 51	6 66
Lignin . . . . .	21 30	11.00	22 91	7 77	6 31
Ash . . . . .	16 90	29.10	45.20	56 90	61 50
Total nitrogen . . . . .	1 18	2 71	1 56	1 77	1 79
Water-soluble nitrogen . . . . .	0 32	1 08	0.14	0 88	0 17
Ammonia-N . . . . .	0 06	0 32	0 04	0.28	0 47

\* 237 days on fresh manure and 215 days on composted manure.

In order to determine how far these principles apply to the growth of the mushroom fungus, which attacks the lignins, in rendering the residual compost a more readily available source of energy for the common fungi and bacteria of the soil, quantities of fresh and composted horse manure were washed with water to remove most of the water-soluble constituents, placed in bottles, sterilized, and inoculated with pure culture spawn of the mushroom. The bottles were incubated for 215 days, then were broken and the material was removed for analysis (table 1). Two-gram portions, on a dry basis, of the fresh and composted manure which had been kept in a sterile condition in bottles, as well as 2-gm. portions of the fresh and composted manure which had been decomposed by the mushroom fungus, were added to a series of 100-gm. portions of fresh soil placed in flasks. The moisture content of the soil was adjusted to optimum for the growth of aerobic organisms and the flasks were connected with the respiration apparatus in the incubator, at 28°C. The amounts of

CO<sub>2</sub> given off daily in the process of decomposition of the various preparations were determined (fig. 1).

In a parallel experiment, 4-gm. portions of the composts were added to 100-gm. portions of the same soil and incubated for 6 weeks. The amount of

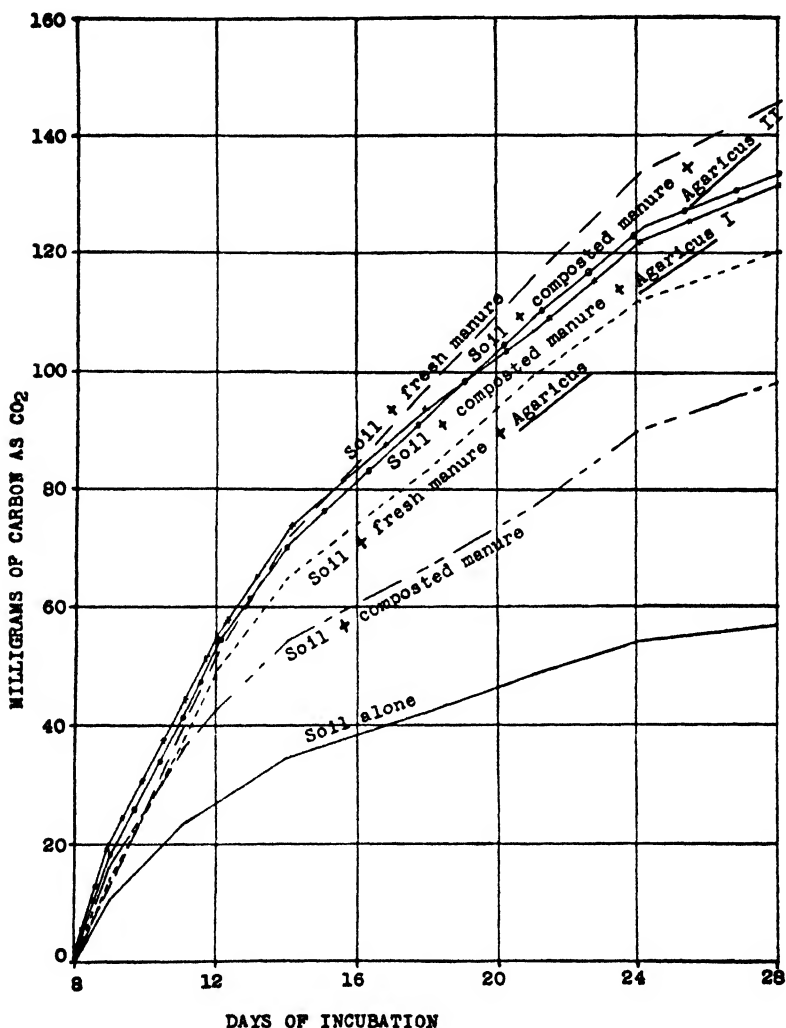


FIG. 1. RATE OF CO<sub>2</sub> EVOLUTION FROM 100-GM. PORTIONS OF SOIL TO WHICH WERE ADDED 2-GM. PORTIONS OF FRESH AND COMPOSTED MANURE, KEPT AS CONTROLS OR IN WHICH THE CULTIVATED MUSHROOM *Agaricus campestris* HAS GROWN FOR 216 DAYS

nitrate produced at the end of that period was determined (table 2). Both sets of results show that in the case of fresh, uncomposted manure, less CO<sub>2</sub> was given off from the sample acted upon by the mushroom fungus and more nitrate was produced than from the corresponding manure in which the mush-



room fungus did not grow but was kept as control. This can be readily understood: fresh manure is rich in readily decomposable carbohydrates, such as hemicelluloses and cellulose, but is low in total nitrogen; as a result of this the rapid decomposition of the carbohydrates, as shown by the  $\text{CO}_2$  liberation, is not accompanied by nitrogen liberation, since whatever nitrogen might be liberated is used by the microorganisms for the synthesis of microbial cell substance. In the case of the same manure in which the mushroom was previously grown, there is a reduction of carbohydrates and a marked increase in nitrogen content, especially the water-soluble form; this is responsible for the lower  $\text{CO}_2$  evolution in soil and greater liberation of nitrogen in an available form.

With the composted manure, totally different results were obtained. The manure itself was decomposed in the soil to a less extent than the corresponding

TABLE 2

*Influence of Agaricus campestris upon making the organic complexes in composts available to soil microorganisms*

NUMBER	NATURE OF COMPOST ADDED TO SOIL	CARBON DIOXIDE LIBERATED*		NITRATE FORMED†	
		Total	Above control	Total	Above control
		mgm. C	mgm. C	mgm. N	mgm. N
1	Control soil	57.3	....	0 97	..
2	Fresh manure, control	146 0	88 7	0	-0 97
3	Fresh manure, decomposed by <i>Agaricus campestris</i>	120 9	63.6	13 75	+12 78
4	Composted manure, control	98.2	40 9	1 42	+0 05
5	Composted manure, decomposed by <i>Agaricus campestris</i> I	132 0	74 7	10.55	+9 58
6	Composted manure, decomposed by <i>Agaricus campestris</i> II	134 1	76 8	11 45	+10 48

\* From 2 gm. of preparations in 20 days.

† From 4 gm. of preparations in 6 weeks.

fresh manure, because in the composted manure there was a reduction of the readily decomposable substances during the process of composting. As a result of decomposition of the composted manure in soil, there was no nitrogen consumed, but a small amount was actually liberated in an available form, as a result of the much narrower carbon: nitrogen ratio in the composted than in the fresh manure. However, when the composted manure was previously used as a medium for the growth of the mushroom fungus it decomposed much more readily in the soil, as shown by the increased  $\text{CO}_2$  evolution and especially by the considerably greater liberation of the nitrogen in an available form. These results prove definitely that composted manure in which the mushroom fungus has been grown, namely the so-called spent mushroom soil, will decom-

pose faster and allow a more rapid liberation of the nitrogen in an available form, but will probably leave much smaller quantities of organic residues in the soil; in other words, spent mushroom soil should be preferable to composted manure as a source of plant nutrients, whereas as a source of humus it should be of inferior value, since it disappears more rapidly than composted manure.

In order to determine what effect the growth of the mushroom fungus has upon the decomposition of other humus complexes, in which an enrichment in lignin and protein has taken place, lowmoor peat was used as a medium. Two-hundred-fifty-gram portions of a lowmoor peat from New Jersey, containing about 50 to 52 per cent moisture, were placed in bottles and sterilized under pressure. Some of the bottles were left as controls and some were inoculated

TABLE 3  
*Decomposition of lowmoor peat by Agaricus campestris*

TREATMENT OF PEAT	TOTAL MATERIAL LEFT	TOTAL WATER-SOLUBLE MATERIAL	TOTAL WATER-SOLUBLE NITROGEN	AMMONIA NITROGEN
	gm.	mgm.	mgm.	mgm.
Sterilized, control peat . . . . .	128 6	1 717	84 2	38.9
Peat in which <i>Ag. campestris</i> was grown for 60 days . . . . .	120 5	3 221	142 0	61 9

TABLE 4  
*Influence of the growth of Agaricus campestris upon the availability of the organic complexes in lowmoor peat to soil microorganisms*

On basis of 30 gm. of dry peat decomposed for 17 days

NATURE OF PEAT	CO <sub>2</sub> LIBERATED	TOTAL WATER-SOLUBLE MATERIAL	TOTAL WATER-SOLUBLE NITROGEN
	mgm. C	mgm.	mgm.
Control peat . . . . .	68 3	277	9.6
Peat in which <i>Ag. campestris</i> was grown . . . . .	107 4	728	32 2

with the spawn of the mushroom fungus. A very fine extensive mycelium of the fungus was produced through the peat, far less abundant than in the case of the composted and even fresh manure used as a medium. After 60 days' incubation, the culture material was removed from the bottles and analyzed (table 3). The results show that as a result of the growth of the mushroom fungus on peat, there was a reduction in the total dry material, accompanied by an increase in water-soluble substances, including nitrogen compounds.

Both the peat kept as control and the peat in which the fungus was grown were now subjected to decomposition by the common microbial flora and fauna of the soil. Fifty-gram portions of the various air-dry peat preparations were placed in flasks, 75-cc. portions of water added, and all the flasks inoculated with a fresh soil infusion. The amount of CO<sub>2</sub> given off during a period of 17

days was measured and, at the end of that period, the water-soluble organic matter, the total nitrogen, and the ammonia nitrogen were determined in the peat cultures (table 4).

As a result of the growth of the mushroom fungus, the peat was rendered more readily decomposable by the common fungi and bacteria of the soil. This is clearly seen by the increase in evolution of  $\text{CO}_2$  and the increase in the solubility of the nitrogen in the peat. This peat is high in nitrogen (about 3 per cent on a dry basis), but the nitrogen is not made readily available as a result of its decomposition by the common microbial population of the soil. However, when the *Agaricus* was grown upon the peat, it produced certain changes in the chemical relation of the organic complexes, which rendered the nitrogen more readily available to the common soil fungi and bacteria.

It was of interest to determine whether the growth of the common mushroom upon composted manure results in a change in the chemical nature of the humus complexes, besides reducing the lignins and rendering the proteins

TABLE 5

*Influence of Agaricus campestris upon the solubility of the humus in composted manure*

MATERIAL	SOLUBLE IN COLD NaOH SOLUTION AND PRECIPITATED BY HCl		SOLUBLE IN HOT NaOH SOLUTION (AFTER COLD) AND PRECIPITATED BY HCl	
	Yield	Nitrogen content of precipitate	Yield	Nitrogen content of precipitate
	per cent	per cent	per cent	per cent
Control .....	4 24	3.37	11 41	3 24
<i>Ag. campestris</i> I .....	9 50	4.00	4 93	2 88
<i>Ag. campestris</i> II.....	9.01	4 30	5 28	3 28

more soluble. The composted manure used in the first experiment, without and with the growth of the *Agaricus campestris*, was treated for 24 hours in the cold with 4 per cent NaOH solution. The extract was removed by filtration and was acidified in the cold with HCl; the precipitate was filtered off, washed free from chlorides, dried, weighed, and analyzed for total nitrogen. The residue left after the cold extraction with NaOH was now extracted with fresh 4 per cent NaOH solution for 1 hour at 15 pounds' pressure. The second extract was also removed by filtration, acidified with HCl, and the precipitate washed and dried. Table 5 shows that as a result of the growth of *Agaricus campestris* upon composted horse manure, there is a definite change in the chemical nature of the humus, as shown by a marked increase in the fraction soluble in cold NaOH solution and the nitrogen content of the precipitated material ("humic acid"): actually three times as much of the nitrogenous complexes were rendered soluble by cold alkali-solutions as a result of the growth of the mushroom fungus upon the composted manure. The fact that this is not due to an increase of this part of the humus at the expense of the

other organic complexes of the compost is shown by the reduction in the fraction soluble in hot alkali solution following the cold. As a result of the growth of the mushroom the humus of the compost was rendered more readily soluble in cold alkali solution.

#### SUMMARY

The growth of the cultivated mushroom, *Agaricus campestris*, upon composted manure results in rendering the organic complexes more readily decomposable by the microbial population of the soil.

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# FURTHER STUDIES ON THE VALUE OF VARIOUS TYPES OF ORGANIC MATTER FOR IMPROVING THE PHYSICAL CONDITION OF SOILS FOR PLANT GROWTH<sup>1</sup>

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Investigations reported previously (3) have shown that three widely different classes of soils were markedly improved for plant growth by the incorporation of various types of organic materials. The improvement obtained from organic additions was largely due to changes in the physical properties of the soils rather than to the nutrients supplied.

Since the experiments on which these conclusions rest were conducted under a system of limited watering, equivalent to about 2 inches of rainfall a month, the results were considered not strictly applicable to conditions of more abundant moisture supply, particularly for clay loam soils having a strong tendency toward unfavorable compaction. Likewise, the questions of the relative persistence of the various types of organic matter in the soil after incorporation, and the changes occurring in the moisture holding capacities of the various organic materials upon drying required further elucidation. Studies of these problems have been made and are here presented.

## IMPROVING THE PHYSICAL PROPERTIES OF A CLAY LOAM SOIL

A critical experiment was conducted during the winter of 1930-31 to determine the relative values of five types of organic substances for improving the physical condition of a clay loam soil, under a system of abundant watering. The soil was practically identical with that used in former studies, having a pH of 5.93, a loss on ignition of 6.5 per cent, a hygroscopic coefficient of 7.2 per cent, a maximum water holding capacity of 36.2 per cent, and an available water holding capacity of 25.7 per cent. The properties of the five types of organic matter have been noted elsewhere (3).

The method of procedure was that followed in former tests, except in the amount of water supplied. In this series of pots (9-inch clay flower pots), 740 cc. of water was added weekly (equivalent to 4 inches of rainfall monthly) to each culture from December 16, after the grass seed had germinated, until

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January 5. The amount was then increased to 1,210 cc. weekly until February 5, to 2,490 cc. until March 3, and to 4,980 cc. until harvest on April 2. The total moisture supplied during this period was equivalent to about six times the quantity used in the previous experiments.

As a result of the more liberal supply of moisture the yields of dried grass were 22 gm. for the untreated soil as compared with 9.6 gm. produced with this soil under a limited system of watering in 1929-30. In spite of losses by percolation, the grass plants showed no signs of wilting in the present experiment, whereas wilting frequently occurred with moisture limited to 2 inches monthly.

The mean daily temperature for the entire growth period was 66°F., and the mean daily water loss from a Livingston atmometer, 105 cc. These values are approximately the same as those noted in the series grown in 1929-30, and are not greatly different from those experienced in the field for the growing season. In general, it may be said that the atmospheric conditions were fully as favorable for the growth of grass as those encountered during the normal growing season of the region.

### *Experimental results*

The effect of incorporating various types of organic matter on the physical properties of the clay loam soils and upon plant growth is shown in table 1. It is noteworthy that the abundant watering produced a satisfactory growth of grass with all treatments, including soil which had received no organic matter. Although the soil was packed firmly in the pots at the time of filling, the drainage was adequate at all times during the experiment, since the plant roots occupied the entire soil mass before compaction from watering had progressed sufficiently to hamper percolation.

An important result was the general increase in plant growth from the use of all five types of organic matter. Furthermore, increasing the amount of organic substance from 73.5 gm. to 147 gm. for every 3,900 gm. of soil, caused nearly as great additional improvement in plant growth as the initial application. With a limited moisture supply, it was observed previously that increasing the quantity of peats reduced plant growth, particularly in the case of imported peat moss. In these instances, reduced growth occurred in spite of improvement in the physical properties of the soil, because of the excessive moisture losses from evaporation and percolation.

The various types of organic matter with abundant moisture respond quite differently from those with limited moisture. Instead of the moderately fibrous Michigan peat showing a distinct advantage over the granular cultivated New Jersey peat, the two substances permitted approximately equal growth with abundant watering. The tardy absorption of water noted for peat moss under conditions of limited moisture supply did not retard plant growth when the moisture supply was abundant.

The relatively high yields of grass on soils receiving spent mushroom soil and manure must be attributed in part to the plant nutrients contained by

these substances. Although sulfate of ammonia was applied periodically during the experiment, this fertilization did not completely overcome the initial differences in nutrient supplying power of the various soil mixtures.

TABLE 1

*The effect of different types of organic matter additions on the physical properties of a clay loam soil, and on yields of grass produced, with abundant watering*

TYPE OF MATERIAL ADDED TO THE SOIL	IGNITION LOSS			AVAILABLE WATER HOLDING CAPACITY*		PORE SPACE† OF SOILS AFTER GROWTH OF GRASS		PERCOLATION OF WATER IN 30 MINUTES	GRASS YIELDS PER POT (DRY WEIGHTS OF TOPS)
	Before growth of grass	After growth of grass	Proportion lost	Before growth of grass	After growth of grass	Actual	Difference from check		
	per cent	per cent	per cent	per cent	per cent	per cent	per cent		
<i>A. Light additions of organic matter—73.5 gm. dry matter to 3,900 gm. moisture-free soil</i>									
Cultivated N. J. peat	7 6	7 2	5 3	30 2	29.7	54.6	+0.1	112	24.7
Raw Michigan peat	7 8	6 9	11 5	27 2	27 7	56 1	+1 6	105	24 1
Imported peat moss	7 8	6 2	20 5	34 1	31 0	61 4	+6.9	121	26.0
Spent mushroom soil	6 8	6 1	10.3	26 0	25 3	53 5	-1 0	94	24 7
Well-rotted manure	7 2	6.2	13.9	27.2	27 9	55.7	+1.2	100	27.7
<i>B. Heavy additions of organic matter—147 gm. dry matter to 3,900 gm. moisture-free soil</i>									
Cultivated N. J. peat.	8 8	8.5	3.4	33.9	31.1	57.1	+2.6	167	26.2
Raw Michigan peat	8 0	7.2	10 0	30 5	29 4	56.2	+1 8	135	26.3
Imported peat moss	9 1	8 2	9.9	39 9	40 2	64.6	+10 1	128	27.5
Spent mushroom soil.	7 0	6 4	8 6	28 8	25 4	52 3	-2.2	90	27.4
Well-rotted manure	8 2	7 1	13.4	34 0	27 4	56 8	+2 3	129	29.5
<i>C. Average of both light and heavy additions of organic matter</i>									
Cultivated N. J. peat	8.20	7.85	4 3	32 0	30 4	55.9	+1.4	140	25.5
Raw Michigan peat	7.90	7 05	10 8	28.8	28 5	56 2	+1.7	120	25.2
Imported peat moss	8 45	7 20	14 8	37.0	35 6	63 0	+8 5	125	26.8
Spent mushroom soil.	6.90	6.25	9.4	27 4	25 4	52 9	-1.6	92	26.0
Well-rotted manure	7.70	6 65	13 6	30 6	27.7	56 3	+1.8	115	28.6
Check (no organic matter added).....	6.5	6 2	4.9	25 7	26.2	54 5	....	107	22.0

\* Available water holding capacity determined by subtracting the wilting coefficient (indirectly determined from hygroscopic coefficient) from the maximum water holding capacity (1).

† Pore space determined with aid of Lebedeff soil sampling tube (2).

Support of this statement is found in the physical properties of the soil mixtures, those containing spent mushroom soil and manure being inferior to those containing some form of peat.



Since the temporary physical effects of organic matter additions to soil are relatively unimportant as compared with the more permanent effects, attention may be centered on the available water holding capacities of soil-organic matter mixtures *after* the growth of grass for the equivalent of a normal season. The improvement in this property is due in part to the incorporation of organic matter with its relatively high water holding capacity, and in part to the improvement in soil structure produced by the presence of organic matter and the effect of the grass roots. Mushroom soil had no lasting beneficial effect on structure because of its relatively low organic matter content (about 24 per cent) combined with rapid decomposition and high soil content. Peat moss proved most effective in raising the moisture holding capacity, followed in order by cultivated New Jersey peat, raw peat from Michigan, and manure.

With regard to pore space, spent mushroom soil was the only material that failed to increase this property of the soil when incorporated therein. Peat moss was the most potent in improving pore space of the clay loam, manure and raw peat followed in degree of effectiveness, with cultivated New Jersey peat nearly as valuable.

Since relatively small changes in total pore space may be accompanied by important changes in structure and differences in water and air movements within the soil mass, rates of percolation for water were determined in the manner described in the previous studies. Cultivated New Jersey peat proved the most valuable substance for increasing the removal of excess water from the soil; peat moss, raw Michigan peat, and manure ranked in order of importance as listed. The spent mushroom soil actually reduced percolation rates.

Determinations of loss on ignition were made as a measure of the relative persistence of the various types of organic matter in the soil. Obviously, the beneficial physical effects of incorporating such substances with soil will continue as long as they are present, and will disappear shortly after the organic content of the soil falls to its former level. The persistence of any organic material is determined by the speed with which it decays after being mixed with the soil. Types of organic matter, such as cultivated peat, that have reached an advanced stage of decay are considerably more resistant to further decomposition than raw peat and peat moss, which have a higher percentage content of readily decomposable compounds. In this connection, it should be recalled that the resistance to decay of the pure substances, is quite different from that exhibited when mixed with soil or fertilizer materials.

Of the five organic matter types tested, cultivated New Jersey peat showed greatest resistance to decay when incorporated with the soil and cropped to grass. Raw Michigan peat disappeared more than twice as rapidly and peat moss three to four times as rapidly as cultivated peat. Well-rotted manure decomposed more readily than raw peat, and less than peat moss. Considering the fact that only one-fourth of the dry matter in mushroom soil was organic in nature, the loss during the growth period of 16 weeks was even greater than for well-rotted manure.

From the standpoint of the persistence of the various types of organic matter, their respective effects on the physical properties of the soil, and the plant growth obtained, the conclusion may be drawn that cultivated New Jersey peat is the most valuable of these types for improving the physical properties of well-watered clay loam soils. Raw peat ranks next in value, followed in order by peat moss, well-rotted manure, and spent mushroom soil. These results are quite different from those obtained with the same material and soil under a limited moisture supply, in which moderately fibrous materials, such as raw peat and manure, were apparently superior.

The application of the results here reported, to field conditions should be made with certain qualifications. Clay loam soils in the eastern United States in many cases are underlaid by a layer of compact soil which may impede sub-drainage. The incorporation of organic matter with the surface layers of soil will not correct faulty structure in lower horizons. In locations where drainage is adequate, the relative value of various types of organic matter for improving the physical properties of the soil for plant growth should be approximately as determined in this experiment. Nutrient deficiencies should be corrected, however, and adequate moisture must be provided for satisfactory results, particularly during the first season of growth after incorporation of the organic matter.

#### EFFECT OF DRYING VARIOUS TYPES OF ORGANIC MATTER UPON THEIR ABILITY TO ABSORB MOISTURE

One of the practical difficulties involving the use of peaty materials for soil improvement is their peculiar relation to water absorption. Although they are recognized as having high absorptive capacities for moisture, layers of these organic substances placed at or below the surface of the soil have appeared to repel water, either allowing moisture to pass through without retention, or restricting water movement and even shedding water as though oiled. A preliminary study of the phenomenon suggested that the explanation lay in the effect of drying on the content of colloidal matter.

An experiment was conducted to determine whether the various peaty substances behaved in the same manner upon drying, or whether they differed greatly in the speed with which the initial water capacity was recovered after being dried and in the extent to which the drying produced an irreversible reaction. In the first tests, the materials were treated in two ways. After being thoroughly mixed, each lot was divided into two equal parts (three for Michigan peat); one part was thoroughly dried in the greenhouse and the other was maintained at its natural moisture content. The peat moss, being obtained in a very dry condition, was divided into two similar lots and one of these was soaked for several days to increase its water content.

All of the materials were then carefully packed into 9-inch flower pots and water was added in equal amounts to each lot of organic matter. The quantity used was equivalent to the calculated maximum water holding capacity of the most absorbent material, namely, peat moss. This amounted to 10.35

kgm. of water for each 1,000 gm. (dry basis) of each type of organic matter. The water was applied as rapidly as it entered the organic matter, and the excess was allowed to drain off. The water content of each substance was then determined, following which an additional application of water was made. After a second analysis, a third addition of water was supplied and a third analysis for moisture content made. The detailed results are given in table 2.

In every instance, drying had an adverse effect on the water holding capacity, even after several times as much water had been added as would completely saturate each substance. In the case of cultivated New Jersey peat,

TABLE 2

*Effect of drying various types of organic matter upon their ability to absorb moisture, when equal amounts of water are applied to all materials*

TYPE OF ORGANIC MATTER TESTED	PREVIOUS TREATMENT	APPROXIMATE MOISTURE CONTENT WHEN TESTED (DRY-BASIS)	WATER ADDED IN EACH ADDITION PER 1,000 GM OF DRY MATTER	MOISTURE HELD AFTER EACH SUCCESSIVE APPLICATION* OF WATER (DRY-BASIS)			RELATIVE AMOUNTS OF WATER HELD AFTER THIRD ADDITION
				After first addition	After second addition	After third addition	
		per cent	kgm	per cent	per cent	per cent	per cent
Cultivated N. J. peat	Natural moisture content	180	10 35	257	282	287	100 0
	Dried in greenhouse	12	10 35	213	243	248	86 4
Raw N. J. peat	Natural moisture content	180	10 35	372	410	439	100 0
	Dried in greenhouse	12	10.35	262	298	308	70.2
Raw Michigan peat	Natural moisture content	150	10.35	241	254	285	100 0
	Dried in greenhouse	12	10.35	147	217	227	79 7
	Dried in oven	.	10.35	75	170	201	70 5
Imported peat moss	Soaked several days	670	10.35	920	953	1137	100.0
	Dried in greenhouse	14	10.35	135	453	600	52 8

\* Equal amounts of water were added to all lots of organic matter, the quantity used for each addition being equivalent to the calculated maximum water holding capacity of the most absorbent material, namely, peat moss.

drying proved least harmful; nevertheless, only 86.4 per cent of the water holding capacity was regained by three successive additions of water. Raw New Jersey peat from the same deposit showed a much lower recovery of absorptive power than the cultivated peat. Raw Michigan peat had a recovery of 79.7 per cent after drying in the greenhouse, as compared with 70.5 per cent following oven-drying. The oven-dried peat was nearly free of moisture at the beginning of the experiment, but the actual water content was not determined. The peat moss lost very little water upon further drying in the greenhouse because its moisture content was already very low. The addition

of three times its theoretical water capacity to each of the two lots of peat moss only succeeded in restoring 52.8 per cent of the capacity exhibited by material after soaking several days.

An additional test on the effect of drying the several types of organic matter was conducted to determine their relative capacities for absorption. In this case, the quantity of moisture added to each material was equivalent to its respective water holding capacity. Three lots of each material were pre-

TABLE 3

*Effect of drying various types of organic matter upon their ability to absorb water when the water added is proportional to the maximum water holding capacities of the respective materials*

TYPE OF ORGANIC MATTER TESTED	PREVIOUS TREATMENT	MOISTURE CONTENT AT BEGINNING OF TEST (DRY-BASIS)	WATER ADDED IN EACH ADDITION PER 1,000 GM. OF DRY MATTER	WATER HELD AFTER EACH SUCCESSIVE APPLICATION OF WATER* (DRY-BASIS)			RELATIVE AMOUNTS OF WATER HELD AFTER THIRD ADDITION
				After first addition	After second addition	After third addition	
		per cent	kgm.	per cent	per cent	per cent	per cent
Cultivated N. J. peat	Natural moisture content	160	2.68	258	278	292	100.0
	Prolonged soaking in water	.	2.68	246	255	287	98.3
	Dried in greenhouse	13	2.68	172	237	247	84.6
Raw N. J. peat	Natural moisture content	294	4.39	493	.	510	100.0
	Prolonged soaking in water	464	4.39	502	507	509	99.8
	Dried in greenhouse	11	4.39	36	107	171	33.5
Raw Michigan peat	Natural moisture content	83	3.46	203	270	271	100.0
	Prolonged soaking in water	227	3.46	243	262	270	99.6
	Dried in greenhouse	11	3.46	29	76	200	73.9
Imported peat moss	Natural moisture content	18	10.35	101	439	528	59.1
	Prolonged soaking in water	...	10.35	821	863	894	100.0
	Dried in greenhouse	11	10.35	109	430	536	60.0

\* The quantity of water added to each lot of organic matter for each addition was approximately equal to the theoretical maximum water holding capacities of the respective types of materials.

pared; one untreated, another soaked in water, and a third air-dried in the greenhouse for comparison with the behavior of the untreated material.

The data presented in table 3 indicate that prolonged soaking did not increase the water holding power of cultivated New Jersey peat, raw New Jersey peat, or raw Michigan peat. Imported peat moss, having a low initial moisture content, was greatly improved by soaking. Peat moss at the normal moisture content of 18 per cent recovered only 59.1 per cent of its potential capacity by three successive additions of the full calculated quantity of water

needed for complete saturation. Further air-drying in the greenhouse did not reduce the absorptive capacity below that of the untreated lot.

Drying produced the least effect on cultivated New Jersey peat, and the greatest on raw New Jersey peat. The behavior of the raw New Jersey peat was unusual in view of its performance noted in table 2. Nevertheless, it is clear that partially decomposed peat is less injured by drying than raw peat, since the cultivated product recovered 84.6 per cent of its water capacity, and raw Michigan peat recovered but 73.9 per cent, as a result of three successive additions of water. This performance was quite similar to that noted for the materials in the test reported in table 2.

In general it may be stated that all of the types of peat investigated were injured in absorptive capacity by drying. The critical moisture content, below which drying should not occur, has not been determined, but thorough air-drying obviously was detrimental. Under field conditions, the tardy absorption of water by dried peaty materials will doubtless greatly increase moisture losses by run-off where such materials are present in considerable quantities as a layer at the surface. Layers of peaty materials placed below the surface are less likely to suffer extreme drying, but may become desiccated in periods of drought. When dried, these layers may be expected to restrict water movement if compacted, and to cause excessive aeration if sufficiently porous to permit the passage of water. In either case the root activities of plants would be unfavorably affected.

#### PERSISTENCE OF FOUR SOURCES OF ORGANIC MATTER WHEN INCORPORATED WITH LOAM SOIL, IN THE FIELD

It is obvious that the value of any type of organic matter for improving the physical condition of soil is determined in part by its persistence upon incorporation with soil. Previous tests (3) conducted in the greenhouse indicated that the organic matter of cultivated peat was most resistant to decay. Raw Michigan peat decomposed more rapidly than cultivated peat but less rapidly than rotted manure. Imported peat moss, although slow to decay in the pure state, decomposed even more rapidly than manure when incorporated with mildly acid soil. When the actual quantity of organic substance present in spent mushroom soil is considered, the rate of decomposition was fully as rapid as that of well-rotted manure. All of these observations, however, were made with soil-organic matter mixtures cropped to grass in the greenhouse, and examined at the end of a period of about 4 months.

Field tests on the persistence of organic matter types were begun in September, 1929, on a well-drained, mildly acid, loam soil. The organic substances were well mixed with the upper 4 inches of soil in 10- by 10-foot plots. Each material was used at the rate of 168 pounds of dry matter per 1,000 square feet of surface in duplicate plots, and also at the rate of 336 pounds in adjoining plots. One untreated companion plot was provided for every set of two plots receiving organic matter additions.

The entire series of plots was planted to a mixture of lawn grasses in September, 1929, and a satisfactory stand of grass was obtained. During the season of 1930 the turf was fertilized with 15 pounds of an 8-6-4 fertilizer per

TABLE 4

*Effect of incorporating various types of organic matter on the organic content of field soils planted to lawn grasses*

TYPE OF ORGANIC MATTER INCORPORATED WITH THE SOIL BEFORE PLANTING*	LOSS ON IGNITION, FOR SOIL PLANTED TO GRASS IN SEPTEMBER, 1929						
	November, 1929		November, 1931		Difference between untreated soil in 1929, and treated soil in 1931	Difference between untreated soil in 1931, and treated soil in 1931	Increase from growth of grass only, 1929 to 1931
	Untreated soil	Treated soil	Untreated soil	Treated soil			
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
<i>Incorporated at rate of 168 pounds dry matter per 1,000 square feet of surface</i>							
Cultivated N. J. peat.....	4.71	4.91	5.02	5.76	+24.4	+14.5	
Peat moss manure .....	4.73		5.09	5.06	+7.0	-0.6	
Spent mushroom soil.....	4.72	4.82	4.97	4.99	+5.7	+0.4	
Well-rotted manure. ....	4.76	5.05	4.89	5.12	+7.6	+4.7	
Average.....					+11.2	+5.05	
<i>Incorporated at rate of 336 pounds dry matter per 1,000 square feet of surface</i>							
Cultivated N. J. peat . . . .	4.71	5.22	5.02	5.57	+18.2	+10.7	
Peat moss manure. . . . .	4.73		5.09	5.62	+18.8	+10.0	
Spent mushroom soil.....	4.72	4.89	4.97	5.02	+6.4	+1.0	
Well-rotted manure.....	4.76	5.05	4.89	5.52	+15.9	+12.9	
Average .....					+14.8	+8.65	
<i>Average for both rates</i>							
Cultivated N. J. peat.....	4.71	5.06	5.02	5.66	+20.2	+12.7	+6.8
Peat moss manure.....	4.73		5.09	5.34	+12.9	+4.9	+7.6
Spent mushroom soil.....	4.72	4.85	4.97	5.00	+5.9	+0.6	+5.3
Well-rotted manure.....	4.76	5.05	4.89	5.32	+11.7	+8.8	+2.9
Average.....					+12.7	+6.75	+5.65

\* The organic materials were mixed with the upper 4 inches of soil in every case.

† The untreated plots were companion plots for the respective treated plots in every instance.

1,000 square feet and in 1931 with 20 pounds. The plots were mowed regularly at a height of three-fourths inch, and watered occasionally during the unusual drought of 1930 to prevent injury to the sward.

Distinct differences in the density and vigor of the turf were noted for the several treatments throughout the two seasons of growth. In general, the most satisfactory development was made on plots receiving the heavy additions of organic matter. The incorporation of the lighter amounts of organic substances, however, permitted a more vigorous growth than that obtained on the untreated companion plots. No weights of grass clippings were obtained, so that quantitative measures of relative growth are not available.

Soil samples were drawn from the upper 4 inches of each plot in November, 1929, and again in November, 1931. Loss-on-ignition determinations were made on these samples as an index of the relative persistence of the various types of organic matter during the 2-year period. The data from duplicate plots were averaged, and the results are presented in table 4.

The outstanding difference between results in the field and those in the greenhouse tests was the fact that loss-on-ignition values displayed a consistent increase in the field plots, while the greenhouse soils showed significant decreases. The average loss on ignition for the eight untreated plots increased from 4.73 per cent to 4.99 per cent as a result of the occupation of the soil by grass roots. Plots receiving organic matter at the beginning of the experiment indicated still greater gains than the untreated companion plots. Presumably the larger increases in soil organic matter arose from greater growth of grass roots following improvement of the physical condition of the soil by the incorporation of the several types of organic matter. The plots receiving heavier additions of organic matter showed greater gains in organic substances as compared with untreated plots, than those receiving the lighter applications.

Comparisons of the effectiveness of the several organic materials for improving the content of soil organic matter should take into consideration the relative loss-on-ignition values of the substances themselves which were incorporated. These values were as follows: cultivated New Jersey peat—88 per cent, peat moss manure—89 per cent, spent mushroom soil—24 per cent, and well-rotted manure—69 per cent. After due allowance is made for differences in actual organic substance added by each type of material, it is clear that cultivated New Jersey peat was the most effective type of organic matter applied. Peat moss manure was less valuable than well-rotted manure, and the organic matter of spent mushroom soil showed the lowest increase in oxidizable material of any substance used.

The difference between spent mushroom soil and rotted manure may be explained on the basis of the rate of decomposition and the percentage of organic compounds resistant to decay, contained by the respective substances. Waksman and Nissen (4) have found that the mushroom fungus utilizes the lignin present in the manure of the mushroom beds more extensively than the cellulose and other readily decomposed constituents. On the contrary, manure undergoing decomposition in the absence of mushroom growth shows an increase in concentration of lignin and a reduction in percentage of readily decomposable constituents. Since lignin is one of the principal substances in

plant residues that resist decay, manure, being comparatively rich in this constituent, decomposes less rapidly than the organic matter of spent mushroom soil. From the standpoint of sustained improvement of the physical condition of the soil, manure therefore is more effective than the organic fraction of spent mushroom soil. For the release of nutrients for plant growth, however, manure may be expected to be less effective, since its rate of decomposition is lower.

The high persistence value of cultivated peat may also be explained on the basis of its relatively high content of lignin and other materials resistant to decay. Peat moss manure, having undergone very little decomposition has a lower content of decay-resisting compounds and therefore decomposes more rapidly than cultivated peat. From the standpoint of their persistence in the soil, the cultivated peat ranks first, well-rotted manure second, peat moss manure third, and spent mushroom soil fourth. With regard to the release of nutrients for use by plants, the order of these substances would doubtless be reversed.

#### SUMMARY

The improvement of the physical properties of a clay loam soil by incorporating various types of organic matter was investigated in the greenhouse with pot cultures planted to grass and supplied with an abundance of moisture. From the standpoint of the persistence in the soil of the several types of organic matter; the sustained improvement in available water-holding capacity of the soil and in its pore space, and the excellence of internal drainage resulting from the incorporation of organic substances; and the growth made by grass on treated and untreated soils, the conclusion is drawn that cultivated New Jersey peat was the most valuable substance included in the tests. Raw Michigan peat was second in value, with imported peat moss, well-rotted manure, and spent mushroom soil following in the order named.

These results were compared with a similar experiment conducted under a system of limited watering, in which the moderately fibrous materials, such as raw Michigan peat and rotted manure, proved superior. With more abundant watering, the rate of decomposition for the raw peat and manure increased, giving the advantage to the more resistant cultivated peat.

Changes occurring in the moisture holding capacities of four types of peaty materials as a result of drying were studied. The drying of each type of organic material to an air-dry condition greatly reduced the capacity for absorption, and this power was recovered very slowly with successive additions of free water. Artificial drying to a moisture content below that obtained in air-drying still further reduced the capacity for moisture absorption.

Cultivated peat was less injured by air-drying than raw peat. Imported peat moss recovered a smaller percentage of its absorptive capacity than any other type of peat, with equal opportunity for moisture absorption. The practical disadvantage in plant production arising from layers of peat below



or at the surface of the soil may be attributed to the tardy recovery of absorptive capacity following extreme drying in droughty periods. It is obvious that peats should be well mixed with the soil mass for improvement of the physical properties of the soil, and that such organic materials should not be air-dry when incorporation takes place.

Comparisons of the relative persistence of four types of organic matter in field soils over a period of two years showed that cultivated peat had raised the organic content more effectively than any other material. Well-rotted manure, peat moss manure, and spent mushroom soil followed in the order given. The rate of decomposition of the organic substance of each of the materials was correlated with the proportional content of slowly decomposable constituents such as lignin.

The respective values of these materials for improving the physical condition of soil over an extended period depend on their relative resistance to decay. The physical value of an organic substance is quite distinct from its value as a source of plant nutrients. In general, the more rapid the decomposition, the greater will be the total quantity of nutrients released. Conversely, the slower the rate of decomposition, the longer will the improvement of the soil's physical condition persist after incorporation of the organic material.

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# THE LAWS OF SOIL COLLOIDAL BEHAVIOR: IX. AMPHOTERIC REACTIONS AND ISOELECTRIC WEATHERING<sup>1</sup>

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The various facts which have led to the present conception of the amphoteric nature of soil colloids have been presented in the preceding articles of this series of papers (16). The soil ampholytoids (amphoteric colloids) may be divided into (a) primary and (b) derived or conjugated ampholytoids.

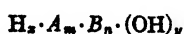
The primary ampholytoids are represented by single compounds such as the hydroxides and oxides of aluminum and iron and by the proteins. The derived or conjugated ampholytoids represent an infinite series of complexes formed by a chemical union between (a) any two or more primary ampholytoids having different isoelectric points (16) and (b) any one ampholytoid and weak acid radicals (12).

The object now is a further study of the manner in which these materials react with simple reagents such as acids, bases, and salts and, conversely, how such reagents influence the formation, the composition, and the decomposition of the amphoteric colloidal complex. The information to be obtained by such a study would, in the first instance, enable us to characterize, by a comparatively simple test, any material of this type under investigation and would, in the second instance, enable us to account for the great variation in composition and for the conditions leading to a "degradation" (pozolization etc.) of the soil complex.

The subject will be divided into two parts of which this, the first, will be of a more general and theoretical nature, whereas the second, which immediately follows, will present the experimental work on amphoteric reactions of soil colloids.

## NATURE OF COMPOUNDS

The derived or conjugated ampholytoids represent compounds of polyvalent weak acids and bases (mostly themselves insoluble) which contain residual H and OH ions by virtue of which they react as acids at high pH and as bases at low pH values. Their general formula might be written:



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<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

where  $A$  and  $B$  represent the anions and cations respectively and  $x$ ,  $m$ ,  $n$  and  $y$  the number of each ion in the colloidal micelle.

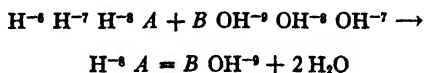
Now the amphoteric behavior of such a complex, i.e., its strength as an acid and as a base, its exchange capacity for cations and anions, its isoelectric point, its point of exchange neutrality, and its ultimate pH (the pH of the free acid-base ampholytoid after complete electro-dialysis) will depend upon several factors. The amphoteric nature of the complex will, in the first place, be governed by the relative as well as by the absolute strength of the acid and basic residue. If the acid residue is strong the basic must be weak and the isoelectric point, the ultimate pH, and the point of exchange neutrality must be on the acid side. If the basic residue is the strongest then the acid residue will be correspondingly suppressed and the aforementioned points will lie on the alkaline side. These points will coincide with the neutral points only when the acid and basic residues are of the same strength (15). If both residues are very weak the amphoteric properties will be indefinite and become manifest only at high acidity and at high alkalinity.

The strength of the acid and basic residue will depend, among other things, upon the relative proportions of the reacting acid and base as well as upon their strength. It is obvious that whenever the reacting materials, such as the acid or base, as well as the products of the various steps in the reaction between them are insoluble there can be no stoichiometric relationship in the precipitate. The strength of the acid and basic residue as well as the acid and basic equivalence in the ampholytoid may therefore vary within wide limits depending upon the relative proportion and strength of the reacting acid and basic radicals. The extent to which neutralization will proceed depends, in addition to the ionization constant of water, upon the ionization constants and concentration of the several acid and basic radicals and upon the solubility products of the resulting combinations. To these must be added the dissociation of the colloid which undoubtedly exists but which cannot be defined in ordinary terms. The composition and properties of the phosphate, silicate, and ferrocyanide series, previously described (19) will serve to illustrate the foregoing.

The various steps in the mutual neutralization of weak, let us say, trivalent acids and bases of a certain type may be illustrated by a few examples. We shall assume, for illustrative purposes, the conditions to be such that neutralization proceeds to the extent that the product of the dissociation constants  $k_a \cdot k_b$  of the strongest remaining acid and basic radicals has been reduced to a value of  $1 \times 10^{-17}$ . The neutralization may of course proceed further if the ionization of the compound formed is small enough. The ampholytoids here studied possess apparent dissociation constants of this order, as indicated by their neutralization curves. But the apparent dissociation constants of colloidal acids and bases must be magnified by the limited dissociation of their salts. The following reactions serve, however, only to illustrate the possible multitude of complexes and, in a general way, to indicate their amphoteric

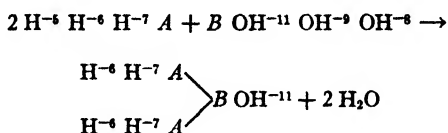
behavior. The negative exponents express the value of  $x$  in the assumed dissociation constants  $1 \times 10^{-x}$ .

Case 1: The acid is stronger than the base but they react in equivalent quantities.



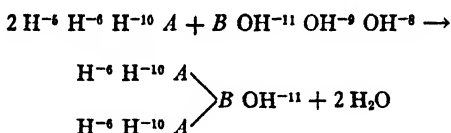
In the ampholytoid the acid residue is stronger than the basic but H is equivalent to OH. The complex is isoelectric and exchange neutral on the acid side. The cation exchange capacity is moderate.

Case 2: The acid is stronger than the base and is present in excess.



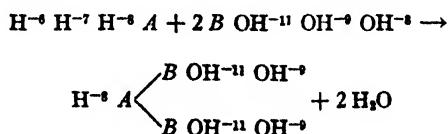
In the ampholytoid the acid residue is much stronger and is quantitatively four times as great as the basic residue. The complex is isoelectric and reacts exchange neutral at a still lower pH than the preceding one. The cation exchange capacity is very high (compare curve I, fig. 23).

Case 3: The same as case 2 except that  $k_a$  of the third H is here lower.



In the ampholytoid the acid residue is much stronger than the basic. Of the four equivalents of H, two are as strong as in case 2 but two are considerably weaker. Since the latter two H ion are too slightly dissociated to be displaced to an appreciable extent by neutral salt cations at pH 7, the cation exchange capacity will be less than in case 2. The isoelectric point which depends upon the strongest H and OH, will, however, be the same (compare fig. 24).

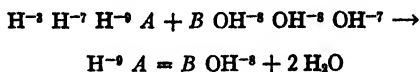
Case 4: The acid is stronger than the base but the latter is in excess.



In the ampholytoid the acid residue is the strongest but the basic residue is quantitatively four times as great as that of the acid. The complex is isoelectric slightly below pH 7.0 and possesses a low cation exchange capacity but

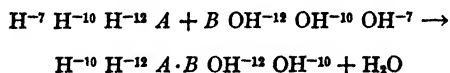
combines with great quantities of anions on the acid side of its isoelectric point (compare curve II, fig. 23).

Case 5: The acid is stronger than the base except with respect to one of the H ions. The acid is equivalent to the base.



In the ampholytoid the basic residue is stronger than the acid residue. The complex is isoelectric and reacts exchange neutral on the alkaline side. The cation exchange capacity is very small if any. (Because of the low dissociation of the exchangeable cations, especially the divalent ones, the H ions may be displaced at a lower pH than that calculated by the buffer equation upon which the curves are based.

Case 6: The acid and base are of equal strength but two of the dissociation constants are very low.



There results then an ampholytoid in which the acid and basic residues are both very weak. The product,  $k_a \cdot k_b = 1 \times 10^{-20}$ , is low and the amphoteric properties are suppressed over a considerable pH range. The complex ought to be isoelectric throughout this range but will, like all inert substances, be electronegative down to a low pH at which the complex combines with a sufficient number of diffusible anions whose dissociation will create a positive charge large enough to balance this "residual" negative charge. (Since even air bubbles are known to carry a negative charge we must ascribe this charge to the water itself; either to an orientation of its molecules or to an unequal distribution of its ions, H and OH, at the interface).

The aforementioned six cases are sufficient to indicate the practically infinite number of combinations. It is obvious that in a colloidal aggregate containing thousands or even millions (11) of molecules almost any relationship as to the strength and quantity of the acid and basic residue is possible. Among the complexes which thus far have been prepared and studied examples will be found which will fit most of the preceding assumed combinations (12).

#### NEUTRALIZATION

In order to obtain an idea of the general form of the neutralization curves to be expected from such combinations and for a comparison with the experimental curves of the various complexes to be shown later (part X) we shall now plot theoretical neutralization curves of the preceding hypothetical combinations. But to be able to do this at all we shall be compelled to use an equation which expresses only in a general way the much more complicated actual relationship. We shall make use of the buffer equation which applies

to the neutralization of a weak acid by a strong base or vice versa. If the salt of a weak acid is completely dissociated the mass law implies the equation (5).

$$[\text{H}^+] = k \cdot \frac{\text{concentration of free acid}}{\text{concentration of salt}}$$

where  $k$  is the dissociation constant of the acid. This equation is only a near approximation unless account is taken of the activity coefficient of the anions of the salt (17). Now, if  $c$  represents the total concentration of free acid plus combined acid, i.e., acid anions, and  $x$  the concentration of the salt, i.e., acid anions, then

$$[\text{H}^+] = k \cdot \frac{c - x}{x}$$

or

$$x = \frac{c}{1 + \frac{[\text{H}^+]}{k}}$$

Henderson (6) believes the law to apply to the neutralization of complex substances like the proteins which contain several acid and basic radicals. If we now likewise assume the equation to apply to our hypothetical complex containing several acid radicals of apparent dissociation constants  $k_1, k_2, k_3 \dots$  and if  $x_1, x_2, x_3 \dots$  represent the concentration of base in combination with them, then the relationships become

$$x_1 = \frac{c}{1 + \frac{[\text{H}^+]}{k_1}}$$

$$x_2 = \frac{c}{1 + \frac{[\text{H}^+]}{k_2}}$$

$$x_3 = \frac{c}{1 + \frac{[\text{H}^+]}{k_3}} \text{ etc.}$$

The total concentration of base in combination with all the acid groups will be the sum of all the  $x$ 's

$$\Sigma x = x_1 + x_2 + x_3 + \dots$$

The corresponding expressions for the neutralization of weak basic radicals by a strong acid become

$$y_1 = \frac{c}{1 + \frac{[\text{OH}^-]}{k'_1}}$$

$$y_2 = \frac{c}{1 + \frac{[\text{OH}^-]}{k'_2}}$$

$$y_3 = \frac{c}{1 + \frac{[\text{OH}^-]}{k'_3}} \text{ etc.}$$

and

$$\Sigma y = y_1 + y_2 + y_3 + \dots$$

where  $y_1, y_2, y_3, \dots$  represent the acid in combination with the basic radicals and  $k'_1, k'_2, k'_3, \dots$  their dissociation constants.

In the combination in the foregoing case 2, the acid dissociation constants  $k_1 = k_2 = 1 \times 10^{-6}, k_3 = k_4 = 1 \times 10^{-7}$ , and the base constant  $k' = 1 \times 10^{-11}$  were assumed. Putting  $c = 1$ , the computation of the quantities of base

TABLE 90  
*Theoretical capacity to neutralize base when  $k_1 = k_2 = 1 \times 10^{-6}, k_3 = k_4 = 1 \times 10^{-7}$*

$\frac{[\text{H}^+]\text{N}}{[\text{OH}^-]\text{N}}$	$10^{-6}$ $10^{-14}$	$10^{-5}$ $10^{-13}$	$10^{-4}$ $10^{-12}$	$10^{-3}$ $10^{-11}$	$10^{-2}$ $10^{-10}$	$10^{-1}$ $10^{-9}$	$10^{-2}$ $10^{-8}$	$10^{-3}$ $10^{-7}$	$10^{-4}$ $10^{-6}$	$10^{-5}$ $10^{-5}$	$10^{-6}$ $10^{-4}$	$10^{-7}$ $10^{-3}$	$10^{-8}$ $10^{-2}$	$10^{-9}$ $10^{-1}$	$10^{-10}$ $10^{-1}$	$10^{-11}$ $10^{-1}$	$10^{-12}$ $10^{-1}$	$10^{-13}$ $10^{-1}$	$10^{-14}$ $10^{-1}$
$x_1$	...	...	...	...	0.01	0.09	0.50	0.91	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$x_2$	...	...	...	...	0.01	0.09	0.50	0.91	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$x_3$	...	...	...	...	...	0.01	0.09	0.50	0.91	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$x_4$	...	...	...	...	...	0.01	0.09	0.50	0.91	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$\Sigma x$	0	0	0	0	0.02	0.20	1.18	2.82	3.80	3.98	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

*Capacity to neutralize acid when  $k' = 1 \times 10^{-11}$*

$y$	1.00	0.99	0.91	0.50	0.09	0.01	...	...	...	...	...	...	...	...	...	...	...	...	...
$\Sigma x - y$	-1.00	-0.99	-0.91	-0.50	-0.07	0.19	1.18	2.82	3.80	3.98	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
$\Sigma x + y$	1.00	0.99	0.91	0.50	0.11	0.21	1.18	2.82	3.80	3.98	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

(x) in combination with the acid radicals and of the quantities of acid (y) in combination with the basic radical at various  $[\text{H}^+]$  yields approximately the values in table 90.

The figures show that  $\Sigma x + y$ , that is, the amount of acid in combination with the acid radical plus the amount of base in combination with the acid radicals of the ampholyte, is at a minimum somewhere between pH 4 and 5. At this point  $\Sigma x - y = 0$  and this should be the isoelectric point, at least if the two salts of the ampholyte are equally dissociated.<sup>2</sup>

<sup>2</sup> But in his mathematical analysis of the problem Michaelis (17) comes to the "very remarkable conclusion" that even if true salt formation takes place on one side or the other, that is, even if the combination with the acid should be less dissociated than the combination with the base or vice versa, the isoelectric point must remain the same. This conclusion

It is clear that if the dissociation constant of the strongest acid radical of the compound in table 90 were increased to  $1 \times 10^{-5}$ , the minimum in the values  $\Sigma x + y$  and  $\Sigma x - y$  would occur at a lower pH, that is to say, the compound would have a lower isoelectric point. A strengthening of the basic radical would have the opposite effect. The formula

$$I = \sqrt{\frac{k_a}{k_b} k_w}$$

where  $I$  represents the isoelectric  $[H^+]$  and  $k_w$  the dissociation constant of water, is another expression for this relation. The isoelectric point of an ampholyte for which  $k_a = 1 \times 10^{-6}$  and  $k_b = 1 \times 10^{-11}$  is then

$$I = \sqrt{\frac{10^{-6}}{10^{-11}}} \cdot 10^{-14} = \sqrt{10^{-9}} = \text{pH } 4.5$$

The compound in table 90 contains two acid radicals for which  $k_a = 1 \times 10^{-6}$ . The isoelectric point of this ampholyte lies therefore somewhat below pH 4.5. This is illustrated by curve I in figure 23, in which the  $\Sigma x - y$  values, which represent the net amount of base (positive values) and of acid (negative values) in combination, are plotted against  $[H^+]$ . The dotted lines represent the separate curves of  $\Sigma x$  and  $\Sigma y$ .

Curve II, figure 23, represents the theoretical neutralization of the combination in the aforementioned case 4. In this compound the basic residue is four times as great as the acid, but the latter radical is stronger than the strongest basic radical. The compound is therefore isoelectric on the acid side, but this point lies in this case only slightly below pH 7.

becomes perhaps comprehensible if we consider that the charge on the ampholyte ions is not alone governed by the dissociation of its salt but also by the dissociation of the remaining undisplaced OH and H. The latter must remain the same at the same pH. Therefore, if an acid or base be added to an ampholyte with which a slightly dissociated compound is formed the only result will be that more of the acid or base will combine at the given pH. The ultimate state of ionization will be the same. In other words the degree of charge (17), which at the isoelectric point equals zero, will be the same, but the point of exchange neutrality, that is, the point at which the ampholyte combines with an equal number of anions and cations will be deflected.

It remains to explain the fact established in a previous article of this series (12) that the more strongly the different anions are adsorbed by the ampholytoid, that is, the less these ions are dissociated by the complex, the greater is the displacement of the isoelectric point to the acid side. Thus the sulfated complex is isoelectric at a lower pH than the chloridated complex, and the phosphated complex is isoelectric at a still lower pH.

The explanation of this influence is that the entrance of these ions in the complex changes its amphoteric nature. Some of the phosphate valences have combined with an equal number of the basic radicals while the remainder of the phosphate valences have added to the strength and number of the acid radicals of the complex whose acid residue has thus been increased while its basic residue has been decreased. In other words, a new complex has been formed in which  $k_a$  is larger and  $k_b$  is smaller. This was shown by an increase in the cation exchange capacity (15).



Figure 24 gives the neutralization curve of the compound assumed in case 3, in which two of the four acid radicals are very weak. This compound will neutralize as much base as the one represented by curve I, figure 23, which has the same isoelectric point, but only at higher alkalinity. Similar neutralization curves are obtained from soil colloids having a high sesquioxide content. Their cation exchange capacity at pH 7 may be very low but at high pH these

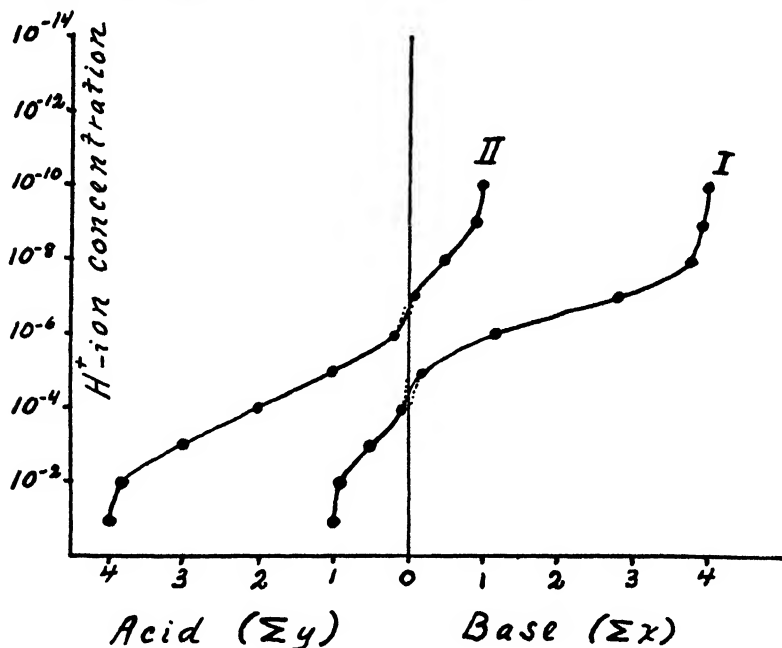
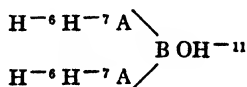
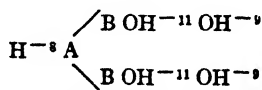


FIG. 23. THEORETICAL NEUTRALIZATION CURVES OF AN AMPHOLYTE

I, of the type



II, of the type



(Negative exponents =  $\alpha$  in the dissociation constants  $1 \times 10^{-\alpha}$ . A and B represent acid and basic radicals respectively.)

colloids adsorb large quantities of bases, as a result of the sesquioxides (10). The amphoteric  $\text{Al}(\text{OH})_3$  and  $\text{Fe}(\text{OH})_3$  are stronger as bases than as acids and have therefore relatively low  $k_a$  values.

The unbroken lines in figure 25 show the theoretical neutralization curve of an ampholyte in which both the acid and basic residues are very weak. The curve represents the compound assumed in the aforementioned case 6. Some proteins as well as certain inorganic ampholytoids yield curves of this type.

## EFFECTS OF LIMITED DISSOCIATION OF COLLOIDAL SALTS

Salts of colloidal ampholytes dissociate apparently to a very limited extent even when the combination is potentially soluble. Thus sodium phosphate, for example, is soluble but the sodium aluminum phosphate complex is only colloiddally dispersible. Each micelle may consist of hundreds of thousands or even millions of molecules. If now all of the Na ions were dissociated by the complex, a potential difference far greater than the assumed critical value of 70 millivolts would be attained (12). We have assumed this to be the cause of

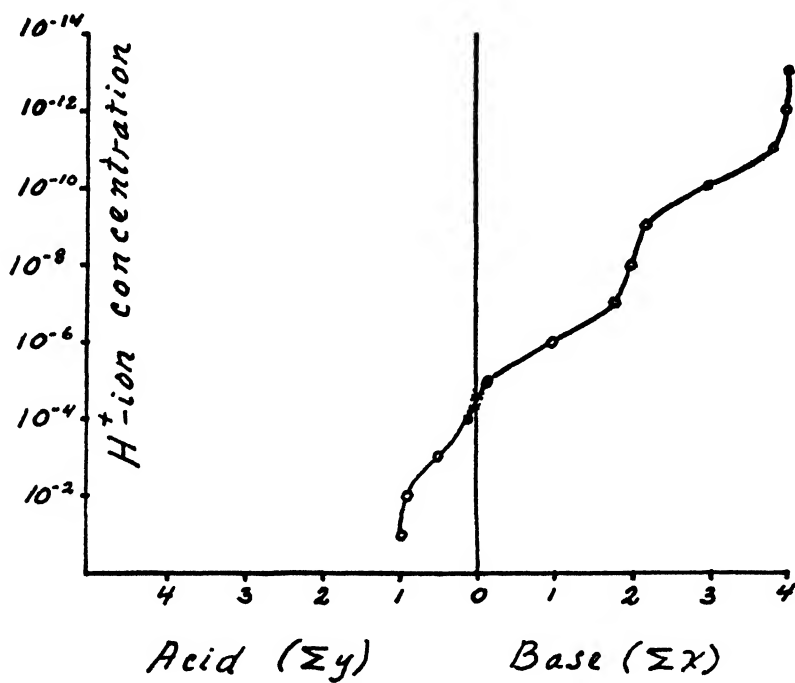
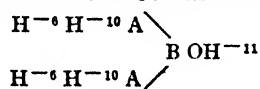


FIG. 24. THEORETICAL NEUTRALIZATION CURVES OF AN AMPHOLYTE OF THE TYPE



the limited dissociation of colloidal salts, which is quite evident, among other things, from the fact that the H<sup>+</sup> ions are displaceable by neutral salt cations.

The effect of this limited dissociation of colloidal salts will be such that the acid and the basic radicals of the colloid will appear stronger the less dissociated the compounds formed with the diffusible cations and anions respectively. Thus a given soil adsorbs more Ca(OH)<sub>2</sub> than NaOH at a given pH and the "unsaturated" soil develops a greater exchange acidity with CaCl<sub>2</sub> than with NaCl (14). The neutralization curves in figure 23 to 25, which are based

on the assumption of complete dissociation of the salts, will, under such conditions, have a different form. The curves will be deflected in the direction of the broken lines as indicated in figure 25. The apparent dissociation constant of the acid and the basic radicals will be greater.

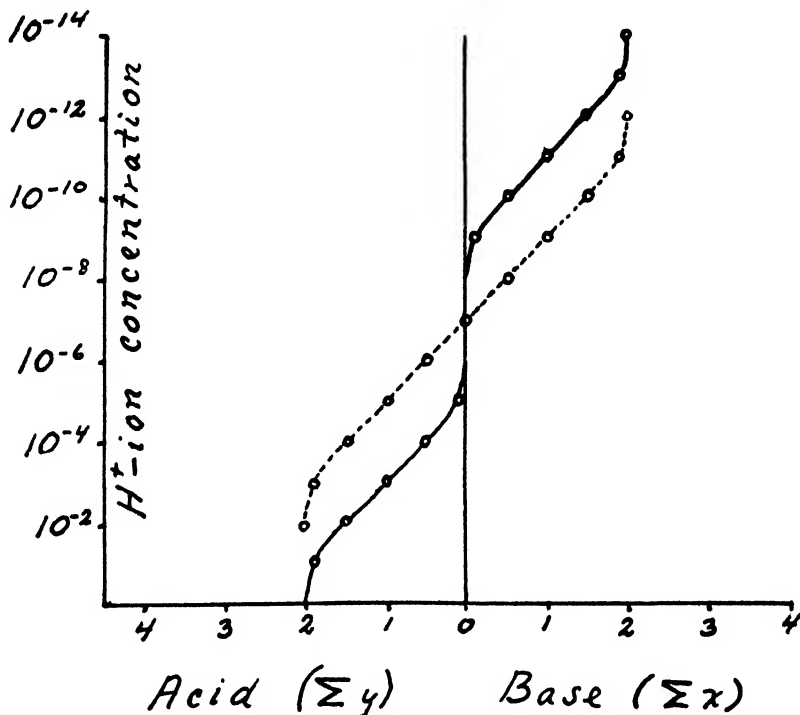


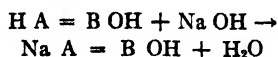
FIG. 25. THEORETICAL NEUTRALIZATION CURVES OF AN AMPHOLYTE OF THE TYPE  $H^{-10} H^{-12} A - B OH^{-12} OH^{-10}$

Broken lines indicate deflection of the curves when incompletely dissociated salts are formed.

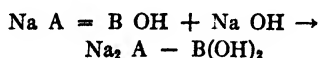
#### HYDROLYSIS

Our scheme of presentation has thus far been based on the assumption that it is only the acid and basic residues of the compounds between weak acids and bases which combine to form salts with strong bases and acids respectively. The compound formed by the partial union of the weak acid and base was considered a stable one. But, except at pH values near the isoelectric point the reaction does not stop here. In solutions of high alkalinity or acidity compounds of this nature undergo more or less extensive hydrolysis. This can be observed by the change in color of certain colored complexes such as ferric "phosphate" and "ferrocyanide" upon the addition of a little alkali.

The hydrolysis of proteins into amino acids is of a similar type. Thus the reaction involving a simple neutralization of the acid residue by a strong base



becomes hydrolytic in the presence of an excess of base, thus



This hydrolysis results finally in a complete decomposition as follows

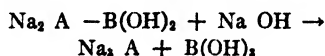


TABLE 91

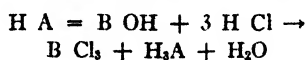
*Theoretical capacity to neutralize base when  $k_1 = 1 \times 10^{-5}$ ,  $k_2 = 1 \times 10^{-6}$ ,  $k_3 = 1 \times 10^{-7}$*

$\frac{[\text{H}^+]}{[\text{OH}^-]}$	$10^{-1}$ $10^{-13}$	$10^{-2}$ $10^{-12}$	$10^{-3}$ $10^{-11}$	$10^{-4}$ $10^{-10}$	$10^{-5}$ $10^{-9}$	$10^{-6}$ $10^{-8}$	$10^{-7}$ $10^{-7}$	$10^{-8}$ $10^{-6}$	$10^{-9}$ $10^{-5}$	$10^{-10}$ $10^{-4}$
$x_1$	.	.	0.01	0.09	0.50	0.91	0.99	1.00	1.00	1.00
$x_2$		..	....	0.01	0.09	0.50	0.91	0.99	1.00	1.00
$x_3$	...	...	..	..	0.01	0.09	0.50	0.91	0.99	1.00
$\Sigma x$		...	0.01	0.10	0.60	1.50	2.40	2.90	2.99	3.00

*Capacity to neutralize acid when  $k'_1 = 1 \times 10^{-8}$ ,  $k'_2 = 1 \times 10^{-9}$ ,  $k'_3 = 1 \times 10^{-10}$*

$y_1$	1.00	1.00	1.00	0.99	0.91	0.50	0.09	0.01	....	....
$y_2$	1.00	1.00	0.99	0.91	0.50	0.09	0.01	....	....	...
$y_3$	1.00	0.99	0.91	0.50	0.09	0.01	.	..	.	...
$\Sigma y$	3.00	2.99	2.90	2.40	1.50	0.60	0.10	0.01	.	....
$\Sigma x - y$	-3.00	-2.99	-2.89	-2.30	-0.90	0.90	2.30	2.89	2.99	3.00
$\Sigma x + y$	3.00	2.99	2.91	2.50	2.10	2.10	2.50	2.91	2.99	3.00

In the presence of an excess of a strong acid the ultimate result would be



The extent to which these reactions proceed depends of course upon the "stability," i.e., the dissociation of the compound.

These reactions play an important rôle in the soil. It is because of this hydrolysis that the cation exchange capacity can be "built up" (14), and the two extreme types of soil transformation, namely podzolization and laterization, represent in all probability nothing but an acid hydrolysis in the first case and a basic hydrolysis in the second case. The following illustration will serve here to show the significance of such hydrolytic reactions.

Assume a mixture of a weak acid and base. Let the dissociation constants of the acid radicals be  $k_1 = 1 \times 10^{-5}$ ,  $k_2 = 1 \times 10^{-6}$ ,  $k_3 = 1 \times 10^{-7}$  and of the basic radicals,  $k'_1 = 1 \times 10^{-8}$ ,  $k'_2 = 1 \times 10^{-9}$ , and  $k'_3 = 1 \times 10^{-10}$ . If we again assume the buffer equation to apply, we obtain the capacities to combine with bases and acids as given in table 91.

If the conditions to which the buffer equation applies, i.e., complete solubility and dissociation of the salt, prevailed, then the neutralization of the acid and the basic radicals would overlap in the  $[H^+]$  range between  $10^{-8}$  and  $10^{-5}$ . This means that the weak acid and base would partly neutralize each other within this range. This salt formation between the two components would attain a maximum in this particular case, at pH 5.5 where  $\Sigma x - y = 0$ .

The ionic equilibria are best seen in figure 26, where the ordinates represent the degree of salt formation, i.e.,  $\Sigma x$  of the acid radicals (A) and  $\Sigma y$ , of the basic radicals (B). The following points are significant:

At  $B^{+++}$  the basic radicals are almost completely neutralized.

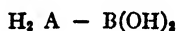
At  $A^{---}$  the acid radicals are almost completely neutralized.

At  $H_2A$  the acid radicals are almost wholly undissociated.

At  $B(OH)_3$  the basic radicals are almost wholly undissociated.

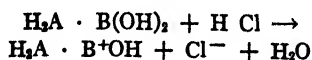
At I there is a maximum combination between A and B.

The acid and basic residue at I is represented by the distances  $IA^{---}$  and  $IB^{+++}$  respectively, which on the ordinate axis correspond to nearly two of the three radicals. The compound is therefore here approximately

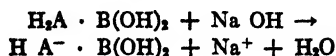


The addition of a strong acid will lead to a salt formation with the basic radicals, which will be almost completely neutralized at  $[H^+] = 10^{-2}$ , whereas the acid radicals pass into the practically undissociated condition at  $[H^+] = 10^{-5}$ . Above this hydrogen-ion concentration there can be no union between A and B. The acid hydrolysis will here be complete. The addition of a strong base will have the opposite effect. The acid radicals will be almost completely neutralized at  $[H^+] = 10^{-9}$  and the basic radicals will be practically undissociated at  $[H^+] = 10^{-8}$ . Below this hydrogen-ion concentration there can again be no compound formation between A and B. The basic hydrolysis will here be complete.

Below a pH of 5.5 the cation  $B^{+++}$  of the basic radicals predominates in the solution whereas at higher pH the anion  $A^{---}$  of the acid radicals is in excess. Now it is obvious that if the compound, let us say  $H_2A \cdot B(OH)_3$ , did not ionize to any appreciable extent or if it were insoluble, that is, existed in the form of a colloidal complex, then the point I would be the isoelectric point. At pH below 5.5 the compound would react with acids, e.g., HCl, and assume a positive charge thus



At higher pH the reaction with bases, e.g., NaOH would yield an anion thus



#### APPLICATION TO INSOLUBLE COMPOUNDS

The formation of an insoluble compound would, however, greatly affect the ionic equilibrium of the system. The foregoing simple relationship has been used solely for the purpose of illustrating reactions which may be quantitatively very different but which are at least of the same nature. The difficulty is that the reactions of colloidal ionogens cannot be expressed in terms of the

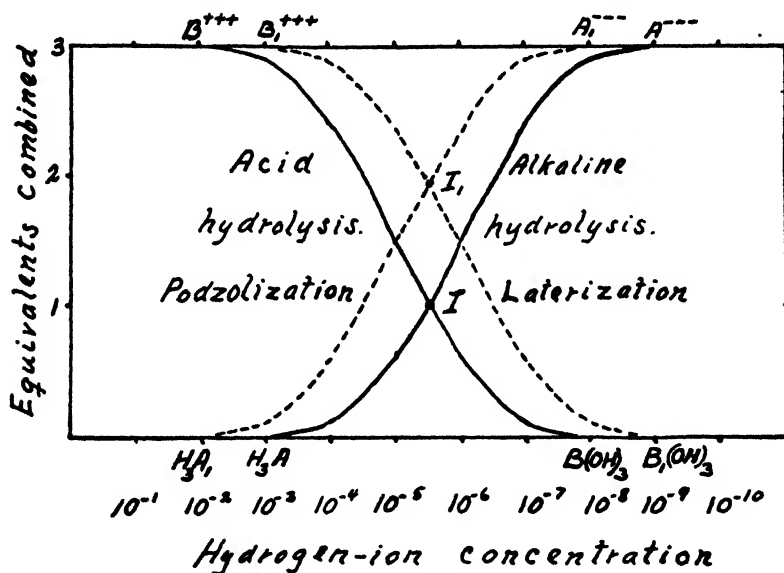


FIG. 26. THEORETICAL NEUTRALIZATION OF A TRI-BASIC ACID WHOSE DISSOCIATION CONSTANTS ARE  $k_1 = 1 \times 10^{-5}$ ,  $k_2 = 1 \times 10^{-6}$ , AND  $k_3 = 1 \times 10^{-7}$ , AND OF A TRI-ACIDIC BASE WHOSE CONSTANTS ARE  $k'_1 = 1 \times 10^{-8}$ ,  $k'_2 = 1 \times 10^{-9}$ , AND  $k'_3 = 1 \times 10^{-10}$

The broken lines indicate deflection of the curves when the acid and the base form a slightly dissociated and insoluble compound. There results then an amphoteric colloid of the type  $\text{H A} = \text{B OH}$  and isoelectric at  $I_1$ .

mass law. Michaelis (17) has analyzed some of the complications arising in the aforementioned dissociation law in cases where undissociated salts are formed with mono- and divalent ions and also in cases of micelle formation, i.e., aggregation of two or more molecules (18). But it would be extremely difficult if at all possible to find a quantitative expression for a reaction so complex as that, let us say, between aluminum hydroxide and silicic or even phosphoric acid.

It is, however, obvious that if a slightly soluble salt is formed the degree of salt formation will be greater the lower the ionic product of the salt and that,

because of the removal of the ions of the salt, the residual acid and basic radicals will be stronger. This is illustrated in figure 26 by the dotted lines which show the direction of the displacement of the neutralization curves under such conditions. The extent of this displacement depends of course upon the ionic product of the compound. The position of  $I_1$  remains, in this particular case, where the acid and base are present in equivalent quantities and where the acid and basic residues maintain the same relative strength, the same as that of I.

The significant thing is also the magnitude of the acid and basic residues which have become much smaller. It is obvious that if the compound between A<sub>1</sub> and B<sub>1</sub> were completely undissociated a normal salt with no acid or basic residue would be formed. This compound would then not react amphoterically except perhaps by hydrolytic cleavage at very low and very high pH values. Also, the greater the acid and basic residues, that is to say, the greater the ion product of the compound, the greater will be the strength of the free radicals, but the compound will be more subject to hydrolysis and therefore less stable.

Among the inorganic compounds studied we have encountered types which correspond to both extremes (13). Thus the ionic exchange reactions of certain "sulfides" (e.g. Sb and Bi) indicate a weak acid residue and great stability, whereas the "sulfide" of tin and especially ferric "ferrocyanide" possess a strong acid residue but are quite unstable even at a pH as low as 7.0 (19).

#### MAXIMUM STABILITY

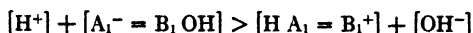
It is clear that the insoluble compound, let us designate it by the formula  $H A_1 = B_1OH$ , is both chemically and physically most stable at the point  $I_1$ , that is, at the isoelectric point or at pH 5.5. There is here a maximum combination between  $A_1$  and  $B_1$ . The number of anions  $-A_1 = B_1OH$  and the number of cations  $H A_1 = B_1^+$  are equal and their sum is here at a minimum. The absence of an electric charge gives rise to an extensive aggregation. The material will become coarse-grained and difficult to disperse. If a solution of, let us say, NaCl of pH 5.5, is added to a suspension of the compound there will be no appreciable change in pH. As a result of the limited dissociation of all salts of colloids there may be some displacement of the OH and H ions by the anions and cations of the salt even at the isoelectric point but this double displacement will be equivalent and the colloid will react "exchange-neutral." [This is only true if the compound formed with the anions and cations of the salt are equally ionized. With NaCl this has been found to be approximately true for many colloids because at the isoelectric point approximately equal quantities of Na and Cl ions were found to be adsorbed. The  $SO_4$  ions, it may be recalled (15), were adsorbed in considerable excess of the Na ions at the isoelectric point. When such differences in "adsorbability" occur it is obvious that the point of exchange neutrality does not coincide with the isoelectric point].

At the isoelectric point the compound will be least reactive, least hydrolytically decomposed, least soluble, and least dispersible.

#### ULTIMATE pH

The reaction of a suspension of the free compound  $H A_1 = B_1 OH$  will be acid since the acid radical is stronger than the basic. Its pH will lie between the neutral and the isoelectric points and will, in concentrated suspensions, approach but never quite attain the latter point. In the free state the compound must be electronegative since more  $H^+$  than  $OH^-$  ions are dissociated. A trace of free acid must be added to increase the ionization of the basic radicals and suppress that of the acid radicals before the compound can be isoelectric. The pH of a concentrated suspension of the free ampholyte is, like the isoelectric point, determined by the relative strength of the acid and basic radicals. To this pH we apply the term "ultimate pH."

Let us illustrate the relationship between the ultimate pH and the isoelectric pH as follows: In a system containing the free compound  $H A_1 = B_1 OH$



or

$$[H^+] > [OH^-]$$

since the acid radical is, in the case considered, stronger than the basic radical.

At the isoelectric point we have by definition

$$[A_1^- = B_1 OH] = [H A_1 = B_1^+]$$

To render the free compound isoelectric we must increase the dissociation of the basic radical by the addition of an acid S which combines with the compound to form  $H A_1 = B^+ + S^-$ .

The system will be isoelectric when

$$[H^+] + [A_1^- = B_1 OH] = [H A_1 = B_1^+] + [S^-] + [OH^-]$$

or when

$$[S^-] = [H^+] - [OH^-]$$

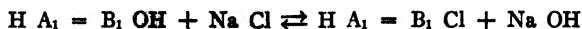
The amount of acid required will depend largely upon the dissociation of the compound which the acid forms with the colloid. The quantitative relationship is therefore different from that of the much simpler systems of soluble ampholytes [comp. Sørensen (21) and Michaelis (17)].

#### EXCHANGE ACIDITY AND ALKALINITY

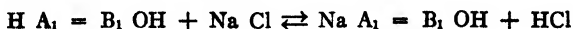
If an acidified solution of NaCl having a pH below that of the isoelectric point is added to the suspension of the free amphoteric colloid it will be found that the pH is increased, that is, acid has been neutralized. The material yields an "exchange alkalinity" on the acid side of the isoelectric point. This



is because the basic radical is here alone reactive and combines with the anion of the salt. If the NaCl solution is less acid than the acidity corresponding to the isoelectric point, an "exchange acidity" will be developed. In this case the acid radicals combine with the cation of the salt. The reactions may be formulated as follows:



at pH below the isoelectric point and



at pH above the isoelectric point.

In the case of soluble and completely ionized salts of ampholytes this neutral salt decomposition could not take place. The explanation of the fact that the reactions are brought about by colloids is that all colloidal salts, as well as the acids and bases, ionize to a limited extent.

The preceding reactions are very useful in the study of the amphoteric soil colloids. Their application will be discussed in the experimental part of this work.

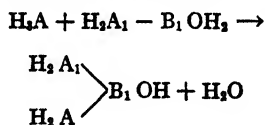
#### ACID AND BASIC HYDROLYSIS

Let us now consider the general behavior of the compound on the acid and alkaline sides of the isoelectric point.

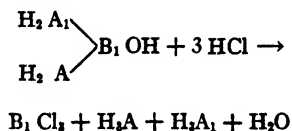
On the acid side the compound will react with the stronger acid responsible for the lowering of the pH. The ionization of the basic radicals will increase whereas that of the acid radicals will be suppressed. The ion  $\text{H A}_1 = \text{B}_1^+$  will predominate and the complex will carry a positive charge and be more dispersible. But at the same time there will be some hydrolytic cleavage of the compound, which ionizes also to a slight extent into  $\text{H A}_1^- - \text{B}_1^+ \text{ OH}$  and  $\text{HA}_1^{--} + \text{B}_1^{++} \text{ OH}$ . There will therefore be formed, first, the ionic species  $\text{HA}_1 = \text{B}^+$  and  $\text{H}_2\text{A}_1 - \text{B}_1^{++}$  and then, at a still lower pH, the ions  $\text{B}_1^{+++}$  and the undissociated molecules of the acid  $\text{H}_3\text{A}_1$ . We shall, therefore, have all degrees of dispersibility down to single ions.

The ionic equilibrium at any one pH will greatly depend upon the nature of the acid with which the compound reacts. Thus it makes a great difference in respect to the dispersibility whether the acid anions are mono-, di-, or trivalent and whether or not they form soluble or slightly soluble compounds with the base  $\text{B}_1 (\text{OH})_3$ . Only monovalent anions would result in a strong positive charge and a maximum dispersion of the complex (11). If the acid is tribasic and therefore also a weak acid and if it forms a slightly soluble compound with the base then there will at first be formed a new complex in which the acid residue is stronger and the basic residue is weaker. This complex will therefore be isoelectric at a lower pH and possess a greater cation exchange capacity, as has been experimentally verified by the phosphated soil complex (15).

Let us illustrate this by the following reaction



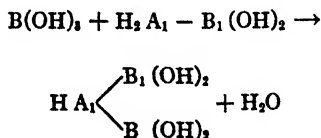
The result is the same if the original compound had been formed with an excess of the weak acid. But even this complex will undergo a hydrolytic cleavage and disintegrate at still a lower pH, thus



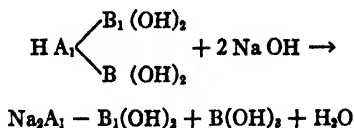
On the alkaline side of the isoelectric point the compound will react with the stronger base responsible for the increase in pH. The ionization of the acid radical will increase, whereas that of the basic radical will be suppressed. The ion  $\text{A}_1^- = \text{B OH}$  will predominate and the complex will carry a negative charge. But here again there will be some hydrolytic cleavage of the compound resulting in the ionic species  $\text{A}_1^- = \text{B}_1\text{OH}$ ,  $\text{A}_1^{--} = \text{B}(\text{OH})_2$ ,  $\text{A}_1^{---}$  and in undissociated molecules of the base  $\text{B}_1(\text{OH})_3$ .

The ionic equilibrium, the dispersibility, and the solubility at any one pH will be greatly modified by the nature of the base with which the acid radicals react. The alkali hydroxides will impart a strong negative charge, a high dispersion, and, at high pH, a considerable solution. The alkaline earth bases, which form slightly dissociated and slightly soluble combinations with the acid radicals, will impart a weak negative charge, a small tendency to disperse, and only a slight degree of solution. The divalent cations will therefore retard the disintegration and the readjustment in the composition of the complex in its otherwise unstable electronegative condition.

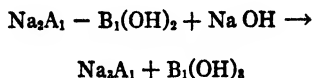
The weak trivalent bases, which form highly insoluble and very slightly dissociated compounds with the acid radicals, will form a new amphoteric complex in which the acid residue is repressed while the basic residue is augmented. The complex will be isoelectric at a higher pH and will have a lower cation exchange capacity. (The trivalent cations in the soil are displaceable by hydrolytic cleavage but not by the neutral salt cations.) The reaction between a trivalent base and the amphoteric compound may be illustrated by the equation:



At high pH, that is to say, in the presence of free alkali, this complex, like the original one, undergoes hydrolysis as follows



and



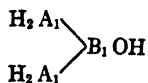
An experimental proof of these reactions was presented in a previous chapter where it was shown that the "aluminated" soil complex is isoelectric at a high pH and possesses a lower cation exchange capacity. The fact that the latter could be partly restored by treating the colloid with alkali points to a hydrolytic cleavage according to the foregoing equations (14).

#### ISOELECTRIC WEATHERING

If we now should attempt to apply these theories to the conditions prevailing in soils, we must first take account of the fact that in the soil, soluble and even colloiddally dispersed materials follow the movement of the water. This means a more or less continuous removal of the soluble or highly dispersible products of a reaction from the insoluble and less dispersible products. It is in general true that materials in the ionized condition are more soluble than in the molecular condition just as colloids are more dispersible in the charged than in the isoelectric condition. It is also true that an amphoteric colloid is least ionized at the isoelectric point.

These facts taken together lead us to the significant conclusion that amphoteric colloids of the type here considered possess a tendency to alter their composition in such a way that their isoelectric point coincides with the prevailing pH. Let us illustrate this statement by two examples.

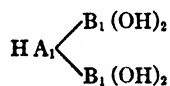
Assume the compound represented in figure 26 (dotted lines) to be subjected to a continuous leaching with a solution at pH 4.5. The basic radicals would be highly ionized at this pH and would, by hydrolytic cleavage, be freed from the insoluble complex and removed by the solution. At pH 4.5 the acid radicals would still possess some combining power and would bind some of the basic radicals. The basic residue would, however, be small whereas the acid residue would be large. Instead of the compound  $\text{H A}_1 = \text{B}_1\text{OH}$  the complex would more nearly correspond to



which, let us say, is isoelectric at pH 4.5. At this point the remaining basic radical would be no more reactive than the acid radicals. They would once more be equally ionized and the complex would be stable, beyond its solubility.

The ultimate result of a prolonged leaching with a solution of a constant pH would, within certain limits, be a complex of such composition as to be isoelectric at the prevailing pH of the leaching solution. The preceding process represents an acid hydrolysis and is represented in nature by the process of podzolization.

Assume now the same original compound to be subjected to a continuous leaching with a solution at pH 6.5. The acid radicals would now be the ones to be highly ionized and would, by hydrolytic cleavage, be freed from the complex and removed by the solution. Here again a stable complex would ultimately be formed at the point where a certain amount of the acid radicals had been lost and where the acid and basic residues once more possessed the same reactivity. The new complex, isoelectric at 6.5, would contain a small acid residue whereas the basic residue would be large. From figure 26 we may assume the composition of the ultimate product of this basic hydrolysis to be approximately



It is clear that if the leaching solution had a still higher pH, let us say 7.5, the ultimate composition of the complex, isoelectric at this pH, would represent a still smaller acid content and a still larger basic content. This basic hydrolysis is represented in nature by the process of laterization.

#### CLIMATE, pH AND COMPOSITION

To summarize let us state our conclusions as follows:

The tendencies in the process of weathering will always be toward the formation of compounds possessing a maximum stability both physically and chemically. This maximum stability, or minimum reactivity, is represented by the isoelectric condition of the ampholytoids. Since now the isoelectric composition of this type of material varies with the pH we should expect a relationship to exist between the composition of the soil colloidal complex and the prevailing hydrogen-ion concentration of the soil solution.

This relationship can, of course, only be fully expressed under conditions of sufficient rainfall to cause extensive leaching and then only in a very general way, because of local differences in climate, geology, etc. Another prerequisite for such a relationship to become manifest is that the climatic conditions must have remained approximately the same for a long period. We shall now look for what evidence we may find.

Let us first briefly review the laws of isoelectric precipitation as experimentally established for the materials in question. Figure 27, which relates

the composition to the isoelectric pH of the aluminum and iron silicate systems, will serve to indicate in a general way what we might expect to happen under natural conditions. The figure is adapted from one in an earlier publication (13).

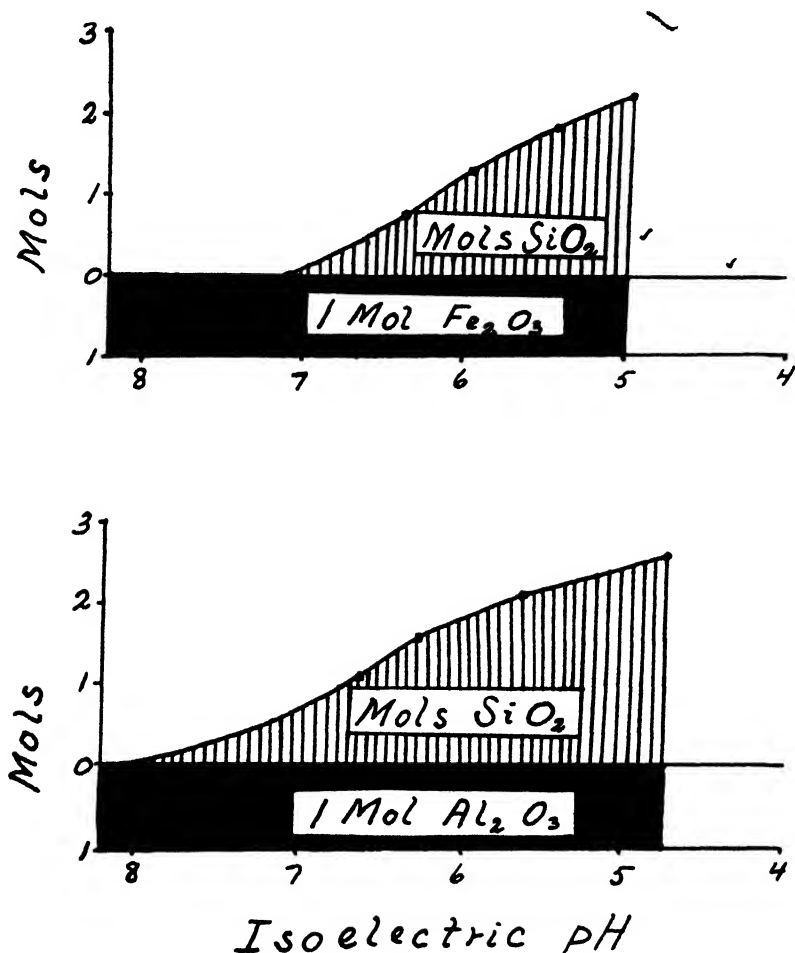


FIG. 27. THE NUMBER OF MOLs OF SILICA ISOELECTRICALLY PRECIPITATED BY 1 MOL ALUMINUM AND FERRIC OXIDE AT VARIOUS pH

#### COMPARATIVE BEHAVIOR OF ALUMINUM AND IRON

It will be noted that the proportion of silicic acid isoelectrically precipitated by aluminum and iron increases as the pH decreases and that aluminum hydroxide, being a stronger base than ferric hydroxide, binds the greatest proportion of the acid at any given pH. Aluminum and ferric "hydroxides" when precipitated from the chlorides are isoelectric at about pH 8.1 and 7.1 respec-

ively. Aluminum "silicate" can therefore begin to form at a higher pH than ferric "silicate."

From this behavior we should expect the silicate of iron to hydrolyze more readily and to be completely decomposed at lower pH than the silicates of aluminum. Of this there is abundant evidence in nature. In the process of laterization, which undoubtedly is conditioned by a nearly neutral or alkaline reaction, the ferric oxide is the first of the amphoteric sesquioxides to be set free. Even in the colder humid regions where we meet with an acid reaction and where, for this reason, the higher silicates of aluminum should be stable, free ferric oxide is always in evidence as shown by the color of the red soils, the ferruginous clays and the brown forest soils of the temperate belt.

As far as the differences in the electrokinetic behavior between the aluminum and iron silicates and the differences we find between them under natural conditions are concerned we find, therefore, a complete general agreement.

#### THE $\text{SiO}_2/\text{R}_2\text{O}_3$ RATIO

The next question concerns the all-important relationship between the composition of the two series and the isoelectric pH. Does this relationship also express itself under natural conditions? To answer this question we must first inquire into the existence of regional differences (a) in the prevailing pH, (b) in the composition of the colloidal complex (for this purpose the composition of the most direct products of weathering as found in the parent material would seem to be more important than the composition of the soil colloidal complex which has been subjected to secondary changes characteristic of the soil body), and (c) in the composition of the drainage waters.

As to regional differences in the prevailing pH no systematic study has been made. But from reports on soil reactions in regions of heavy rainfall and in the absence of carbonates (the necessary conditions for our present study) it appears that the soil reaction in tropical regions where the conditions favor laterization is more nearly neutral whereas in the colder regions an acid reaction prevails. That this should be so can easily be explained. In the tropics, the weathering is much more intense. Quantities of strong bases are liberated by hydrolysis. The organic matter is rapidly oxidized into very weak carbonic acid and water. The reaction of the soil solution, especially when it reaches the lower zone of weathering on top of the parent rock, will remain neutral or even slightly alkaline in spite of heavy leaching. Under such conditions the hydroxides of aluminum and especially iron possess little or no power to bind silicic acid. The silicates undergo here a rapid alkaline hydrolysis (comp. fig. 26) the soluble products of which (the silica and the strong bases) will be leached out.

In the colder humid regions the weathering is much slower. Smaller quantities of strong bases are liberated within a given interval of time. The organic matter is transformed into acids much stronger than carbonic. The result is a more acid reaction. Under these conditions aluminum and, to a lesser

degree, iron will combine with silicic acid. The proportions of silicic acid thus fixed must increase within certain limits with increasing acidity, as shown in figure 27. [At low pH, aluminum and iron become ionically mobile as single and complex cations, that is, the complex undergoes an acid hydrolysis brought about by humic acids which displace the silica (13). This is pozolization which gives rise to a bleached layer of very high acidity and in which the remaining small amount of silicate complex should, and has been found to (1), possess a high proportion of silica. In the podzol B horizon the pH is higher. The aluminum and iron would therefore not be able to combine with as much silica as in the more acid A horizon but this is not the chief reason for the low ratio of silica to sesquioxides in the B horizon. The aluminum and iron exist here chiefly in combination with humic instead of silicic acid. The formation of this complex must therefore not be identified with laterization, as some would believe. The latter process consists of an alkaline hydrolysis, whereas podzolization is the result of an acid hydrolysis. The laterites possess little or no cation exchange capacity because they contain a low proportion of silica and practically no acid residue. The complex of the podzol B horizon may possess a considerable acid residue, due to humic acid, and accordingly a high cation exchange capacity.]

If now the reaction of the percolating water in humid regions changes from neutral in the tropics to acid in the colder zones we should anticipate an accompanying change in the composition of the products of weathering somewhat similar to the relationship shown in figure 27. On the one hand, i.e., in the tropics, we should expect the colloidal complex to possess a very low ratio of silica to sesquioxides, and on the other, i.e., in the podzolic regions of the North we should expect to find a more or less highly silicated colloidal material even where practically all of the strong bases have been lost through leaching. The intermediate zones would be expected to show an intermediate composition.

The meager data so far available support in a general way the predictions here made. The various publications of the Bureau of Soils workers (1, 7, 8, 20) represent the most valuable contributions to this subject. Byers and Anderson (3) have very recently published a general summary on the composition of the various soil colloids investigated in the bureau. From this work the writer has computed the average ratio of silica to sesquioxides in the soil colloids from the humid states of the Atlantic seaboard as far as represented. Table 92 gives this average for each state in the order of its latitude from Cuba to the St. Lawrence basin. The Cecil series are especially interesting because they occur on a wide stretch extending from Alabama to Virginia. We are therefore able to compute the composition ratio of the colloids from a single soil series as found in widely different latitudes.

The figures represent the average composition of the A, B, and C horizons. Whether this average composition ratio or the ratio in any one of the different horizons best represents the composition ratio characteristic of each locality cannot be decided. With the exception of the podzols the different horizons

do not differ greatly in composition. In the podzol B horizons the silica sesquioxide ratio is often very low because of the precipitation of "humates" of aluminum and iron. In such cases the ratio of silica to sesquioxides loses its significance. As already pointed out (15) the amphoteric behavior of the complex is a function of the ratio of the activity of the acid residue to the activity of the basic residue. In the podzol B horizons the  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratio may be very low whereas the ratio  $\frac{\text{acid activity}}{\text{basic activity}}$  may be high. Any relation-

ship between pH and composition must of necessity take account of all the acid and basic radicals present in the complex. To attempt this here would introduce complications beyond the scope of our present study. It should be pointed out, however, that the colloids from the Beckett horizons all contain a high humus content which undoubtedly has displaced silicic acid. This applies perhaps also to the Chester series, which are classified as gray-brown podzolic soils.

TABLE 92

*The ratio of silica to sesquioxides in soil colloids from different latitudes of humid regions*

COLLOID	LOCALITY	NUMBER OF ANALYSES	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$
Nipe.....	Cuba	3	0 30
Cecil.....	Alabama	3	1 27
Cecil.....	Georgia	9	1 33
Cecil.....	North Carolina	9	1 44
Davidson.....	North Carolina	4	1 44
Cecil.....	Virginia	3	1 60
Chester.....	Maryland	22	1 61
Leonardtown.....	Maryland	12	2 01
Podzols:			
Beckett.....	Massachusetts	5	1 74
Emmet.....	Michigan	3	2 93
Superior.....	Wisconsin	4	2 94

The table shows a definite, if not very great, increase in the composition ratio of the red Cecil colloids from Alabama to Virginia. The differences become very great as we examine the two extremes, i.e., the brick red Nipe laterite and the podzol colloids. Great local variations will undoubtedly be found. Such variations may be due to several causes affecting the prevailing pH. Thus the pH may be lowered by the oxidation of pyrites etc., or it may be increased by infiltration of waters from lime deposits. Variations in the humus cover and in the drainage are other factors. The nature of the vegetation is another factor which greatly affects the pH and the process of weathering.

A very interesting example of this latter influence has been brought out by Lundblad in Sweden (9), who points out that the brown forest soils of southern Sweden undergo a rapid "degradation" or podzolization in cases where the native hardwoods (beech) are succeeded by conifers (Norway spruce).



The relatively high pH under the hardwoods has given rise to a complex which possesses a comparatively low ratio of silica to sesquioxides. Under the more acid conditions following the growth of spruce this complex is no longer stable. Its isoelectric point is too high for the new environment and this leads to an acid hydrolysis resulting in a new complex with a higher ratio, which alone is stable at the lower pH.

$$\text{THE } \frac{\text{SiO}_2}{\text{MO} + \text{M}_2\text{O}} \text{ RATIO IN COLLOIDS AND IN RIVER WATER}$$

If the processes of weathering follow the laws of isoelectric precipitation we should expect to find evidence of this in the composition of drainage waters. The composition of the water which carries away all dissolved materials should be complementary to the composition of the insoluble residue—the colloidal complex. The composition of the water should even prove a surer index than the composition of soil samples, provided the waters to be compared represent humid regions which differ chiefly in the position of latitude.

The composition of soil colloids may vary greatly as a result of local conditions. The material may be recently formed or it may be very old, and formed under different conditions. Then we never know to what extent the colloidal fraction as separated by the super-centrifuge consists of unweathered particles of the original minerals. Glacial "rock flour" may yield colloidal material of this type. The composition of the drainage water must, on the other hand, constitute a most direct expression of what is taking place within the zone of weathering at the present time and represent the most complete general average obtainable.

In this connection our problem is simplified by the fact that we are not concerned with absolute values but merely with a ratio between certain constituents. It is clear that if the increase in the silica/sesquioxide ratio of soil colloids as we go from the tropics to colder regions is the result of a greater power of aluminum and iron hydroxides to combine with silicic acid as the pH decreases northward, then we should find a greater relative proportion of silica to the strong bases in tropical waters than in the waters of colder regions. The strong bases, especially Ca and Na, are rapidly lost as the minerals hydrolyze under humid conditions. Some of the silicic acid is, anionically, dispersed or dissolved together with the bases. How much of the silica which will thus pass into dispersion or solution will depend upon the extent to which the silicate ions are freed, by hydrolytic cleavage, from their union with Al and Fe and this will depend upon the pH.

Clarke (4) states that in natural waters "silica is actually present in the colloidal state and not in acid ions." Such distinctions are too sharply made. The colloidal state does not exclude ionization. A colloidal micella possesses all the characters of an ion and represents apparently an aggregate of ions and undissociated molecules. The degree of dispersion of the insoluble substance appears indeed to be closely related to the number of free ions (11). The ionization of the silicic acid will depend on the pH (comp. fig. 26) and will not

be wholly suppressed in the presence of, no matter how much,  $\text{CO}_2$ . It seems that too much emphasis is placed on the action of  $\text{CO}_2$  in relation to the hydrolysis of silicate rocks. Theoretically we should expect a more rapid hydrolysis of silicates in a slightly alkaline than in a slightly acid solution since these materials are isoelectric on the acid side or, in other words, since the acid radicals are stronger than the basic radicals.

From data supplied by Clarke (4), and representing an average of about 1,700 analyses, the following figures on the composition of the igneous rocks have been computed. They express the percentage as well as the relative number of mols of four of the strong bases and of silica. Putting  $\text{CaO} = 1$  we find:

	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SiO <sub>2</sub>
Per cent . . . . .	4.83	3.74	3.05	3.37	59.83
Mol ratio . . . . .	1.0	1.08	0.37	0.63	11.48

This makes a ratio of  $\frac{\text{SiO}_2}{\text{MO} + \text{M}_2\text{O}} = 3.73$

The corresponding figures for 44 of the soil colloids from various parts of the United States as reported by Robinson and Holmes (20) are as follows. (The Houston colloid is here omitted because of its  $\text{CaCO}_3$  content):

	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SiO <sub>2</sub>
Per cent . . . . .	0.96	1.72	1.43	0.38	43.35
Mol ratio . . . . .	1.0	2.50	0.89	0.35	41.90

The ratio of silica to the sum of the bases becomes in this case  $\frac{\text{SiO}_2}{\text{MO} + \text{M}_2\text{O}} =$

8.85

It is evident by comparing the two series of values that the bases are leached out in relatively greater quantities than the silica. The loss appears to be in the following order:



The soil colloids studied by Robinson and Holmes represent soils of all degrees of maturity. If we compute the same ratio for the colloids of highly mature soils, such as the Cecil series, we obtain a different picture. The following figures are calculated from the 33 analyses of Cecil colloids as reported by Holmes and Edgington (8). These samples include the 24 whose silica/sesquioxide ratio is given in table 92. The authors give the pH values of the original soils. These values are also given in table 93. We shall find it instructive to give the average values for each state represented as well as the average values for the entire series.

In comparing the highly matured Cecil colloid with the colloids from various parts of the United States we note that: (a) All percentages of silica and strong bases have become lower but the loss of bases is by far greater than that of

silica. (b) All ratios have become wider. To 1 mol of CaO there are here 2.76 mols MgO, 1.67 mols K<sub>2</sub>O, and only 0.33 mols of Na<sub>2</sub>O, whereas the SiO<sub>2</sub> has increased (relatively) to 34.8 mols per mol of strong base. (c) In the different states the ratio of silica to strong bases increases as we go southward. (d) The pH of the original soils increases likewise in the same direction.

Before discussing these points we shall present, for comparison, the composition of river water from different regions.

The following ratios have been computed from the composition of river water, as given by Clarke (4). The rivers selected include those of the St. Lawrence Basin, of the Atlantic slope from Maine to Georgia, and of British Guiana. This represents a fairly continuous humid belt extending from the far North to the equator. The rivers have been placed into the same groups as given in Clarke's tables. The average values for each group were then calculated.

TABLE 93  
*The ratio of silica to strong bases in the Cecil soil colloids*

LOCALITY	NUMBER OF SAMPLES	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SiO <sub>2</sub>	SiO <sub>2</sub> MO + M <sub>2</sub> O	pH
		per cent	per cent	per cent	per cent	per cent		
Virginia.....	3	0 08	0 62	0 56	0 07	39 41	27.2	4 53
North Carolina ...	10	0 18	0 33	0 51	0 05	36 52	34 2	4 90
Georgia.....	17	0 18	0 30	0 46	0 06	35 77	35 6	5.02
Alabama.....	3	0 13	0 23	0 32	0 09	35 17	45 6	5 23
General average:								
Per cent. ....		0 17	0 33	0 47	0 06	36 30	.. ..	..
Mol ratio .....		.. ..	.. ..	.. ..	.. ..	.. ..	.. ..	....
CaO = 1. ....		1 0	2 76	1 67	0 33	200 0	34.80	....

The composition of many of the waters represents the mean of a great number of analyses. The division is made into the following groups which include the lakes and rivers named:

I. St. Lawrence basin.

Lakes: Superior, Michigan, Huron, Erie, Champlain.

Rivers: St. Lawrence, Pigeon, Grand, Kalamazoo, Maumee, Genesee, Oswegatchie, Ottawa.

II. Maine to Pennsylvania.

Lakes: Moosehead, Rangeley.

Rivers: Androscoggin, Merrimac, Hudson, Raritan, Delaware, Susquehanna.

III. Maryland to North Carolina.

Rivers: Potomac, Shenandoah, James, Don, Roanoke, Neuse.

IV. North Carolina to Georgia.

Rivers: Cape Fear, Pee Dee, Saluda, Wateree, Savannah, Ocmulgee, Oconee.

V. British Guiana.

Rivers: Barima, Waine, Essequibo, Demerara, Courantyne, Potaro.

For details the reader is referred to Clarke (4, p. 68).

It is very significant that the ratio of silica to strong bases increases rapidly as we go south from the St. Lawrence to the equator. The increase is from 0.19 to 1.55, or about 8 times in the latter region as compared to the former. Our object now will be to account for these observations.

If isoelectric weathering is a fact, if the laws of isoelectric precipitation govern the processes in nature as they do in the beakers of the laboratory, we shall have no difficulty in accounting for what we have observed because there is nothing which the theory does not demand. Everything that we have observed might, in a general way, have been predicted.

Let us first consider the interesting relationship between the pH, the composition, and the locality of the Cecil series of colloids (table 93). If the pH, as found, represents the prevailing, or at least the order of the prevailing, pH then we should expect the silica content of the colloid to be highest in the north

TABLE 94  
*The ratio of silica to strong bases in river waters of different latitudes*

LOCALITY OF RIVERS	MOL RATIO. CaO = 1.0:					$\frac{\text{SiO}_2}{\text{MO} + \text{M}_2\text{O}}$
	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SiO <sub>2</sub>	
St. Lawrence basin . . . . .	1 00	0 45	0 03	0 17	0 31	0 19
Maine to Pennsylvania . . . . .	1 00	0 34	0 07	0 49	0 55	0 29
Maryland to North Carolina . . . . .	1 00	0 39	0 04	0 35	0 84	0 48
North Carolina to Georgia . . . . .	1 00	0 35	0 13	0 92	2 10	0 88
British Guiana . . . . .	1 00	0 99	0 15	1 90	6 28	1.55

For comparison we write again the same ratios for:

Igneous rocks . . . . .	1 00	1 08	0 37	0 63	11 48	3 73
44 soil colloids . . . . .	1 00	2 50	0 89	0 35	41 90	8.85
Cecil colloids . . . . .	1 00	2 76	1.67	0 33	200 00	34 80

where the pH is lowest (comp. fig. 27). Where the pH is higher the aluminum and iron silicates become more highly hydrolyzed, i.e., the silicate ions are displaced by OH, resulting in a liberation, dispersion, and solution of silica. More silica is therefore lost by the complex in the southern states. This is, however, true only when the rainfall is heavy enough to remove the strong bases, especially the divalent Mg and Ca which themselves form slightly soluble compounds with silicic acid and which are powerful coagulants of negative colloids. In soils of arid and semi-arid regions and also in the case of young soils or deposits in humid regions there can be no relationship between pH and composition. The colloids in such soils have usually a high silica/sesquioxide ratio in spite of a high pH but then their content of strong bases is always high (20).

The relation between pH and composition of the Cecil colloid might seem paradoxical when we consider the ratio of silica to strong bases. The lower

this ratio, that is, the higher the proportion of strong bases, the lower is the pH (see table 93). Before the amphoteric nature of the complex and its relation to the ultimate pH had been revealed this phenomenon could not have been understood. The fact is that the highly leached Cecil colloid is nearing an equilibrium condition. Its pH is not far from its ultimate pH. The small amounts of bases still left make little difference. The pH is dominantly determined by the silica-sesquioxide ratio and must therefore be highest where this ratio is lowest. A sample of Cecil colloid having a  $\text{SiO}_2/\text{R}_2\text{O}_3 = 1.34$  yielded, when electrodialyzed, a pH of 4.75. Its isoelectric point was somewhat lower (15). The Cecil colloids studied by Holmes and Edgington and cited in tables 92 and 93 were nearly unsaturated. The pH must therefore be highest where the  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratio is lowest. A colloid of the order of the Cecil might be almost unsaturated at, let us say, a pH of 5.0, whereas another colloid having a very much higher ratio might be 50 per cent or more saturated at the same pH (15).

It thus appears that although the ultimate equilibrium composition (probably never quite attained) is governed by the prevailing pH, the effect in turn becomes the cause in that the pH is regulated by the composition.

If we now turn to a study of the ratios between the individual strong bases in the colloids we note, as already pointed out, that these ratios widen as the leaching progresses, being widest in the highly weathered and matured Cecil colloids. As far as the bases are concerned this widening in the ratios between them must be ascribed to a difference in stability of the compounds formed with the alumino and ferric silicates. It is well known that whereas a large part of the Ca (sometimes all of it) exists in the exchangeable condition only a very small part of Mg and K in soil colloids are subject to displacement by neutral salt cations. Mg and K appear to predominate in the interior of the particles, perhaps because they fit better into the ionic lattice and into the formation of slightly dissociated compounds. Mg and K would therefore be locked up in the interior of the particle during its formation from the products of hydrolysis. On the surface of the particles the relative proportions of the different cations are more like the composition of the soil solution (or river water) if we are to judge by the proportion in which the cations are exchangeable (2).

The widening of the ratio between the strong bases reflects the relative stability of the compounds which these bases form with the colloidal complex. Since practically all the strong bases are ultimately lost this difference in stability cannot affect the ultimate product of isoelectric weathering. But where a high stability retards the loss of a base it will also retard the loss of silica and thus postpone the final equilibrium condition.

Regarding the widening in the ratio of silica to strong bases in the colloids as compared to the igneous rocks the explanation is, on the basis of our theory, quite obvious. Below their isoelectric pH the sesquioxides combine isoelectrically with a certain proportion of silica, the proportion depending upon the

pH (see fig. 27). At the isoelectric point the amphoteric complex combines neither with anions nor cations to any appreciable extent. Therefore if the system tends to become isoelectric it is clear that the observed widening in the ratio between silica and strong bases must result.

Besides this widening in the ratio of silica to strong bases in the colloids as compared to the igneous rocks we also note a widening in the same ratio as the silica/sesquioxide ratio decreases or as we go from the north to the south, as is seen in the case of the Cecil colloids in table 93. As the silica content of the colloid decreases southward the strong bases decrease at a still higher rate. This is due to a very rapid decline in the strength of the acid residue as the silica content decreases. It will be recalled that the base exchange capacity is not a linear function of the number of mols of silica (or acid radical in general) present in the complex but that this capacity increases or decreases much more rapidly than the corresponding increase or decrease in the silica content (14). This means that the greater the proportion of sesquioxide the smaller is the free silicate mol fraction or acid residue and the smaller, therefore, the base-combining capacity per mol silica. In the laterites the often not inconsiderable quantities of silica possess hardly any combining power. Hence the widening ratio of silica to strong bases.

If we now examine the ratios of silica to strong bases as found in the river water we note that the ratios are, in a general way, complementary to those found in the soil colloids. The ratios found in the igneous rocks occupy a middle position (see table 94).

In respect to these ratios it should be pointed out that the presence of lime and other similar deposits may greatly alter the "normal" ratio which would result from the weathering of igneous rocks in each of the different regions. There are, of course, many other factors which will affect the ratios, such as differences in composition of the igneous as well as the sedimentary rocks. The sodium in many of the rivers near the ocean represents to a large extent cyclic salt. The nature and magnitude of a multitude of such disturbing factors are too difficult to trace and will not here be further discussed.

The quantities of sesquioxides present in the river water of the various regions are small and fairly uniform, as far as reported, with the exception of the brown waters of British Guiana which contain in some instances rather large quantities of  $\text{Fe}_2\text{O}_3$ . This iron is believed to have nothing to do with the actual process of weathering but rather to represent a secondary product resulting from the action of humus on the lateritic complex in the South American swamps. No account is therefore taken of this fact. It should also be pointed out that the humus has nothing to do with the high silica content in these waters, for, as Clarke states (4), the silica is equally high in the small streams near their sources.

The most significant thing about the composition of the drainage waters of the different regions is the ratio of silica to strong bases. The increase in this ratio from the St. Lawrence basin to British Guiana at the equator is so great

that all local irregularities can well be ignored. The differences must be due directly or indirectly to the climate. If the differences are the result of an isoelectric weathering then they depend upon differences in the prevailing pH and this in turn depends upon the climate.

In the St. Lawrence basin the pH must be low. The sesquioxides combine, therefore, with a greater proportion of silica. There is, therefore, on the average, only 0.19 mols  $\text{SiO}_2$  going into solution with each mol  $\text{MO} + \text{M}_2\text{O}$ . As we go southward the prevailing pH increases, the OH ions take more and more the place of the silicate ions in the aluminosilicate and ferric-silicate complex. The result is that the ratio of silica to strong bases increases in the drainage waters.

At the equator the soil reaction becomes more nearly neutral. Under these conditions ferric hydroxide combines with little or no silica whereas aluminum hydroxide unites with some silica even in a slightly alkaline medium. Very much greater quantities of silica will therefore be dissolved out together with the strong bases. In the river waters of British Guiana the ratio of silica to strong bases reaches a value of 1.55. This is still less than half of the same ratio in the igneous rocks, which is 3.73, but the difference is not so great if account is taken of the fact that a large part of the silica in the rocks consists of highly insoluble quartz.

Laterization is undoubtedly the dominant form of weathering in British Guiana. This is evident not only from the high ratio of silica to strong bases but also from the ratios between the individual bases which have become more narrow and approach the ratios in the igneous rocks. (The high value of 1.90 for  $\text{Na}_2\text{O}$  may be ascribed to cyclic salt from the ocean.) The laterites, and also bauxite, may contain considerable quantities of  $\text{SiO}_2$  but they are, perhaps without exception, notorious for their poverty in strong bases. The low percentage of bases in the laterites is due to an extremely weak acid residue. The widening in the ratio of silica to strong bases observed in the case of the Cecil colloids and referred to in the foregoing attains a maximum in the laterites. The latter retain hardly any of the strong bases. The result is that the ratio between the bases in the drainage water will be more nearly that of the igneous rocks.

In closing it might be pointed out that isoelectric weathering is a tendency rather than a fact. It must not be assumed that the products of hydrolysis necessarily unite to form an isoelectric complex at the very outset. The isoelectric complex must possess the maximum stability but it is not the only stable compound. Comparatively high stability may extend over a wide range on either side of the isoelectric point. In combination with slightly dissociating ions, e.g., with divalent cations, the electronegative soil complex possesses a high stability and may resist decomposition for a long time even at a pH far removed from the isoelectric point. There must, however, even then be a gradual, if slow, trend toward an isoelectric composition. If the bases are lost more rapidly than they are returned to the soil (by man or by vegetation) this trend will be much more clearly manifested.

The same tendency toward the formation of an isoelectric complex might exist in the organic colloids as well. From the laws governing the isoelectric precipitation of proteins by humic acids and by the sesquioxides, as established by the author (16), we might conceivably account for certain facts such as the high percentage of nitrogen in the organic matter of the B horizon together with many other similar observations. This problem is, however, more complicated and cannot be discussed before certain questions, now investigated, have been answered.

In the next paper it will be shown how different soils and how the materials from different soil horizons react amphoterically.

#### SUMMARY

If an insoluble weak base as represented by  $B(OH)_3$  reacts with a weak acid, as represented by  $H_3A$ , to form an insoluble compound then we may have



or any intermediate combination.

These compounds contain acid and basic residues of varying strength and react amphoterically. Where the acid residue is weak, as in (a), the isoelectric pH will be high and where the acid residue is strong, as in (c), the isoelectric point will be at a low pH. This relation between the composition and the isoelectric pH, which has previously been experimentally verified, has been applied to a study of the process of weathering. It is in general true that amphoteric compounds are least dispersible, least soluble, and least ionized, and are therefore most stable at their isoelectric point. Therefore, under conditions of heavy leaching, the tendency would be for the formation of an isoelectric soil complex.

A study of the prevailing pH in different latitudes of humid regions, of the composition of soil colloids and of river water supports this theory of isoelectric weathering.

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# SOME IMPORTANT SOIL PROFILES OF SOUTHERN PUERTO RICO

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The information which appears in the body of this report was obtained in connection with the soil survey of the Ponce area, Puerto Rico, during the winter season of 1929-30.

The accompanying map (fig. 1) of Puerto Rico (10) shows the principal physiographic features of the island, and figure 2 shows in greater detail the physiographic features of the Ponce area.<sup>2</sup> This area, on which the soils were studied in detail, lies largely between Ponce and Salinas and between the Caribbean Sea and the foothills of the main mountain mass of the island. It embraces about 150 square miles. Brief reference is also made to other parts of the island for purposes of comparison.

## CLIMATE

Puerto Rico lies well within the tropical zone and being far removed from large land masses, has a distinctly oceanic climate (2). The trade winds which blow almost constantly from the northeast bring large quantities of moisture, and the upward deflection of these winds by the mountains of the island causes a heavy precipitation on the northern and central parts of the island (fig. 3). As the winds descend toward the south coast they are adiabatically warmed and tend to take up rather than deposit moisture. The Ponce area is then, much drier than the northern and central parts of the island. Great local variations of rainfall in the southern coastal plain are demonstrated by the records for Juana Diaz and Hacienda Potala, which are only 3.4 miles apart. The former receives 51.65 inches and the latter only 28.79 inches. The maximum precipitation for the area is probably about 60 inches and the minimum is probably less than 25 inches.

The average annual temperature of the Ponce area is approximately 78°F. with an annual variation from this average of not more than 1°. The diurnal

<sup>1</sup> The author is especially indebted to Mr. Guillermo A. Torruella for his assistance in the field, and to Director R. Fernandez Garcia and to Mr. Jose H. Ramirez of the Insular Experiment Station, for assistance in the laboratory investigations. Dr. N. L. Britton, of the New York Botanical Gardens and author of many articles and books on the flora of Puerto Rico and elsewhere, made valuable contributions by identifying numerous plant specimens collected in connection with the studies of the solonchak soils.

<sup>2</sup> Thorp, J., and Torruella, G. A. 1930 Soil Survey of Ponce Area. (Unpublished preliminary report to be embodied later in Soil Survey of Porto Rico).

variation is seldom more than  $20^{\circ}$ . The highest temperature recorded for the island is  $100^{\circ}$  at Ponce; the lowest recorded is  $41^{\circ}$  in the high mountains (2).

#### VEGETATION

Corresponding to the great variation in rainfall in the Ponce area there is a comparable variation in vegetation. The sandy coast line, where well drained,

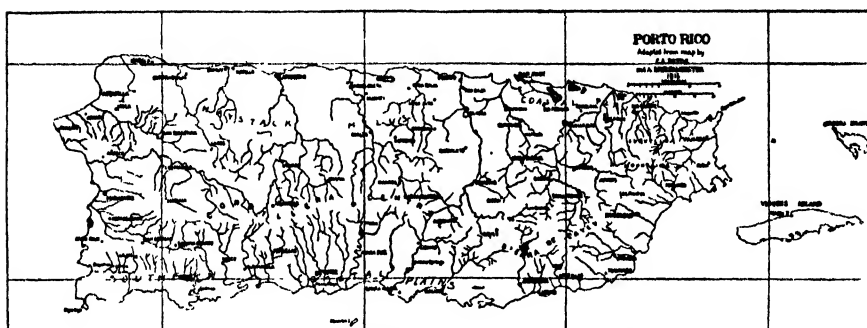


FIG. 1. MAP OF PORTO RICO

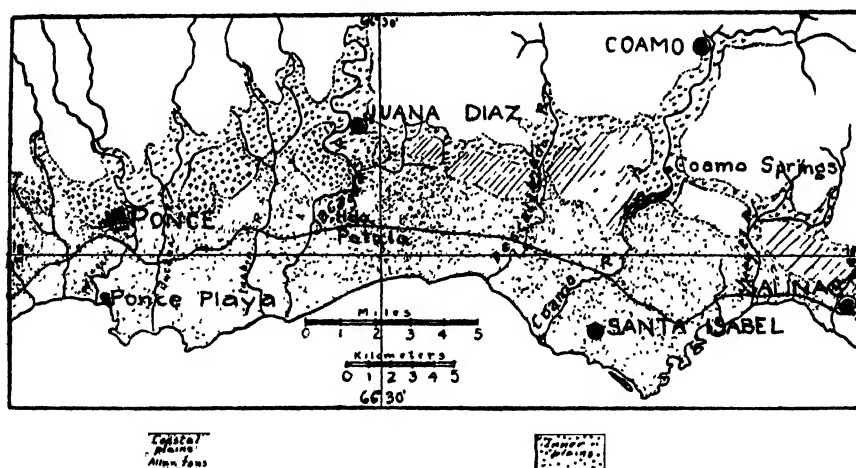


FIG. 2. PHYSIOGRAPHY OF PONCE AREA  
(After Lobeck)

supports a xerophytic growth of cactus, chaparral, and grasses. Poorly drained areas near the seacoast (and elsewhere) are likely to contain salts and alkali and when this is the case they support a halophytic type of vegetation. The front range of soft limestone hills is covered by a xerophytic chaparral which has, in places, been cleared and planted to guinea grass for pasture. The coastal and inner plains have been so largely cleared and cultivated that

it is difficult to determine the original type of vegetation. From a few nearly virgin areas observed the normal vegetation of these areas appears to be a combination of xerophytic grasses and a scattering of trees, of which the bucar is one of the most common. These grasses extend well up on the convex slopes of the foothills, whereas mesophytic forest growths cover the moister concave slopes of the adjacent ravines (1).

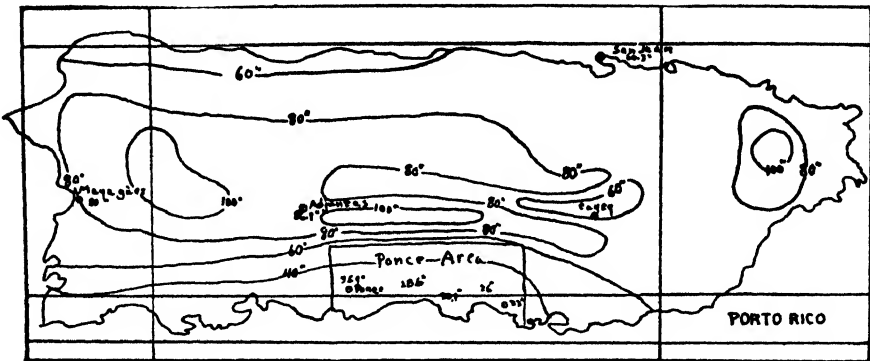


FIG. 3. RAINFALL OF PORTO RICO IN INCHES  
(Data from the U. S. Weather Bureau)

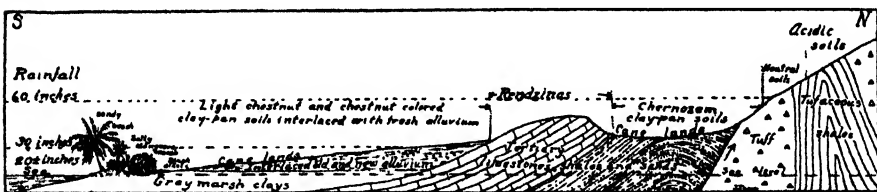


FIG. 4. IDEAL CROSS SECTION IN PONCE AREA NEAR JUANA DIAZ SHOWING RELATIONSHIP OF SOILS TO CLIMATIC AND GEOLOGICAL FACTORS

Not drawn to scale. Geologic section adapted from Mitchell (8)

#### SOILS

One of the first questions asked by the average soil investigator is "Did you see any laterites in Puerto Rico?" or, "Are all soils in Puerto Rico lateritic?" We may unreservedly answer "No" to the last question, about which more will be given later. There is a fairly large area of ferruginous laterite on the Mayagüez mesa and at a few other points on the island. Fettke and Hubbard (5) describe the limonite deposits on the Mayagüez mesa as being residual in origin and offer analyses of the deposit and the underlying serpentine as proof. These deposits are described as being practically identical to those found in northeastern Cuba. The deposits in Cuba are described by Marbut (7) as true laterites. Mitchell (8) studied the laterite deposit on Mayagüez mesa

and confirms the opinions of Fettke and Hubbard regarding it. Sweet (11) describes lateritic soils found in the San Juan area and elsewhere in his paper read before the American Soil Survey Association in 1929. Our purpose is to describe a group of tropical soils, which because of climatic differences, are in no way related to the laterites and lateritic soils briefly mentioned in the foregoing.

In mapping the soils of the Ponce area after the usual manner of the Bureau of Chemistry and Soils, approximately 29 series comprising 90 types and phases were separated. This great multiplicity of series, types, and phases can be directly attributed to the complexity of the environmental factors of climate, geology, drainage, and, to a lesser extent, vegetation. The reader will not be interested in the tiresome details of the points upon which these separations were made. We propose, therefore, to describe briefly a few profiles of well-drained mature soils, the general characteristics of the rendzinas and the general conditions existing among the poorly drained and solonchak soils.

TABLE 1  
*Base exchange N  $\text{NH}_4\text{Cl}$  of the heaviest layer of four "clay pan" soil types\**

SOIL TYPE	DEPTH	S. N. NUMBER	LABORATORY NUMBER	$\text{R}_2\text{O}_3$	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O
	<i>inches</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Ponceña clay	12-16	580502	8107	0 0000	1 32	0 055	0 013	0 020
Santa Isabel silty clay loam . .	6-12	580526	8108	0 0025	0 94	0 064	0 009	0 080
Paso Seco loam	16-28	580570	8109	0 0000	0 68	0 075	0 010	0 016
Fe clay .	10-22	580558	8110	0 0000	0 97	0 074	0 016	0 022

\* Analyst G. Edgington, August 12, 1931.

Since all of the maturely weathered soils of the Ponce area have most of the morphological characteristics of the true solonetz type, one may say that a normal or zonal profile is non-existent there. Base exchange investigations (table 1) indicate that from a chemical standpoint, as interpreted by the Russian soil scientists, these soils cannot be classed as true solonetz. In order to avoid confusion, therefore, we shall refer to them as "clay pan" soils.

There are at least two types of these "clay pan" soils corresponding to climatic differences. One of these is the chernozem-"clay pan" soil and the other is the chestnut-colored "clay pan" soil. The former occurs under a rainfall of from approximately 40 inches to approximately 60 inches. The chestnut-colored "clay pan" soil occurs under rainfalls varying from about 25 inches to about 40 inches. Possibly one might find a few areas of light chestnut-colored "clay pan" soil in the Ponce area. Opinions differ so widely on colors and degrees of darkness of color that we hesitate to make positive statements.

*Profile 1*

Profile 1 was taken from the unplowed margin of a smooth, gently sloping cane field 1 kilometer northwest of Coto Laurel. This field lies on the inner plains between the range of limestone hills and the foothills of the mountains. It receives an annual precipitation of 45 to 50 inches. Virgin areas are very small and are covered chiefly by pasture grasses with a scattering of various kinds of trees. A description follows:

HORIZON DEPTH inches	HORIZON DESCRIPTION
0-12	Very dark brown to black clay. Highly granular, the grains being fine near the surface and gradually increasing in size with depth. Very plastic when wet. No effervescence with HCl. Many coarse and fine roots.
12-16	Dark brown, <i>very heavy and tough</i> clay mottled with black clay from above horizon. It is the heaviest layer of the profile and forms large, very hard columnar clods when dry. No effervescence with acid. Roots less abundant than in the above horizon.
16-26	Yellowish brown, <i>heavy and tough</i> , roughly columnar clay with black soil from the surface horizon in irregular vertical tongues following the soil cracks. No effervescence. A few roots.
26-30	Light yellow-brown silty clay with a few white, soft lime concretions. Interstitial soil effervesces with acid.
30-54	Yellow-brown silty clay mottled brown, red, yellow, and white. The latter is due to lime, which is in one-fourth to one-half inch concretions, soft on the outside and hard in the middle. This is the horizon of maximum lime accumulation. There are many roots.
54-66	Shaly limestone (or limy shale) mottled yellow, reddish yellow, and gray. Effervesces freely with acid. Texture, silty clay loam. This is the parent material and it shows distinct lines of stratification. It seems to have much less lime than the horizon above it.

Since the first profile, described to represent the chernozem—"clay pan", is derived from a shaly limestone it was thought best to describe another soil of the same group in order to prove that the dark color of the soil and the accumulation of the lime are not due simply to the nature of the parent rock. For this purpose a soil of another series is described in the following. This was taken from a high alluvial terrace about 3 kilometers east of Juana Diaz plaza and just north of the Military Road. The alluvial materials consisted of tufaceous shale fragments, basic igneous gravels, and occasional hard limestone fragments. It will be noted that this soil differs in broad characteristics from profile 1 in having its lime accumulation at a greater depth.

*Profile 2*

DEPTH inches	DESCRIPTIONS OF HORIZONS
0-2½	Dark gray-brown, very fine granular silty clay, matted with grass roots.
2½-10	Dark gray-brown clay, nearly black when wet. Forms large granules and small clods which subdivide with difficulty into a medium granular structure. Many areas of this soil are practically black in this horizon.
10-18	Very heavy, tough, cloddy, roughly columnar brown clay. Heaviest horizon of

- profile. There are some angular gravels, many grass roots in the cracks, and many vertically distributed dark-colored soil deposits in worm holes.
- 18-30 Light brown clay loam, heavy, porous, and cloddy. Much angular gravel. No effervescence with acid.
- 30-42 Same as above with a slight effervescence with acid and with a larger proportion of gravel.
- 42-60 Interstratified layers of gravel, sand, and silty lime deposits, the latter strongly predominating.
- 60-72 Loose stratified gravel and sand. Little lime present.

In many places this profile is underlain by a series of buried soil profiles, each with its horizon of lime accumulation. The predominate color of these buried soils is reddish or purplish. Profile 2 supports a xerophytic vegetation, chiefly grasses.

Nearly all of the chernozem-"clay pan" soils are on the inner plains. The chestnut-colored "clay pan" soils lie chiefly on the broad alluvial fans which make up a large part of the coastal plains. There are several series of these soils, but a description of one or two will suffice.

### *Profile 3*

DEPTH inches	DESCRIPTIONS OF HORIZONS
0-6	Dark brown, coarsely granular silty clay loam, turning light brown when crushed.
6-12	Dark brown (lighter colored when crushed), very heavy and tough silty clay. Contains a few angular lime concretions but the interstitial soil does not effervesce.
12-18	Brown silty clay loam full of hard angular lime concretions and worm holes filled with dark brown material from above. Slightly compact.
18-36	Alternating layers of yellowish brown fine sandy loam and silt loam with many spots of soft lime, some small iron rust stains, and some gray mottling. Iron streaks are very abundant at the bottom, in some places forming a hardpan.
36-40	Yellow-brown fine sandy loam to loamy sand with some gray and rusty mottlings and occasional limy spots. Often this layer does not effervesce with acid.

Profile 3 was taken from the edge of a cane field on the seacoast  $2\frac{1}{4}$  kilometers west of Pastillo. The A horizon has been somewhat disturbed by plowing operations. Different members of this series show considerable variation in the thicknesses of the different horizons.

Another "clay pan" profile which occurs in fairly large areas of the coastal plain lacks the usual horizon of lime accumulation. It seems probable that this may be due to lack of sufficient bases in the parent materials to cause the formation of lime. The chief parent material seems to be quartz sands. It may also be possible that the more porous nature of the soil has permitted leaching to extend to a greater depth. A sample of this soil, taken from a high alluvial terrace just northeast of Barrio Paso Seco, is described in the following.

### *Profile 4*

DEPTH inches	DESCRIPTION OF HORIZONS
0-8	Dark brown loam, coarsely granular, with granules which break up fairly easily into single grains.

- 8-16 Dark brown (yellow-brown when crushed) silty clay loam, coarsely granular to finely cloddy.
- 16-28 Heavy, brown, *very tough* silty clay, columnar in form. There are many insect burrows filled with dark-colored organic material from above. The soil is yellow-brown when crushed.
- 28-44 Light brownish yellow, cloddy, somewhat tough loam to fine sandy loam. Columnar form. There is some dark-colored material in the worm holes.
- 44-60 plus Light brownish yellow loamy fine sand; structureless.

This sample came from a road cut and was in a semi-virgin condition. Grass roots were especially abundant in the two upper horizons. Natural vegetation on this type is xerophytic.

Another soil with a very similar structural profile, but having a nearly black A horizon was found to have unsymmetrical lime concretions about 1 inch long and  $\frac{1}{2}$  inch thick between the depths of 4 and 5 feet. These concretions appeared to have been partly dissolved and there was no interstitial lime in the soil. This sample was observed on a high terrace in the southwestern edge of the city of Juana Diaz.

Mature soils of the Ponce area have the following striking characteristics in common:

A thick (10 inches-15 inches), heavy textured (with few exceptions), granular A horizon, gray-brown to nearly black in color, according to climatic conditions.

An extremely heavy, columnar, prismatic, or blocky B horizon containing vertical streamers of dark-colored material washed in from the A horizon.

A horizon of lime accumulation (with the exception of one series), the lime occurring both in concretions and in the silty or crypto-crystalline forms.

With the exception of profile 1, and a very few others not here described, the mature soils are derived from old alluvial fan and high terrace deposits of varying composition.

In a few areas near the soft limestone hills, notably near Central Mercedita, there are a few rendzina-like soils. Their A horizons range in color from brown to black. They can scarcely be classified as true rendzinas because there is usually a feeble development of the heavy B layer which characterizes the "clay pan" soils. This development is never very pronounced, however. These soils are underlain by either a soft chalky limestone or by alluvial material consisting entirely of outwash from the soft limestone hills. In places where the outwash from the soft limestone hills is mixed with material from the igneous rocks of the interior, the profile more nearly approaches the true "clay pan" type.

The poorly drained soils of southern Puerto Rico are derived mainly from lagoon and estuarine deposits which lie at or very near sea level. The soils of both the old and recently deposited alluvial fans abut on these filled-in lagoons and estuaries and are poorly drained near the line of contact. In the virgin condition these soils range from pale gray to nearly black at the surface and all of them except a few areas of peat and muck have predominately gray subsoils. In a few places lime concretions similar to those described in



profile 3 of the mature soils are encountered at depths of from 1 to 3 feet. These concretions seem to represent the average position of the water table. With the exception of peat and muck most of the poorly drained soils contain lime from the surface downward. It is usually in the form of sea shell fragments. In the poorly drained soils the water table is encountered from the surface to depths of 5 or 6 feet. Some places are marshy at all times whereas at others there may be water at the surface part of the time with the true water table lying at 30 inches or more.

Some of the best cane soils of the south coast are those which are of recent alluvial origin (3). Their lack of definite profile characteristics makes them uninteresting from a purely scientific viewpoint. They lie on the same alluvial fans as some of the mature soils, and, because the river courses sometimes change, these soils often interlace with those showing mature development. Most of these soils will effervesce with dilute HCl from the surface downward because of the inclusion of quantities of ground-up land-snail shell fragments. These shells are also frequently found in the A horizons of the mature soils.

A common characteristic of all the soils of the region, excepting those which are swampy or heavily impregnated with salts, is brought about by the very great activity of earthworms and burrowing insects, especially the former. Myriads of deposits brought from the deep subsoils are deposited in the upper horizons and great quantities of material are also carried down from the surface to the B and C horizons. This activity of the earthworms is greater than we have ever observed in any other group of soils.

Land crabs are very active in the poorly drained soils. They burrow from the water table to the surface and build chimneys of gray mud on the surface like those of the crawfish in the Southern States. They are sometimes a serious pest in the cane fields and are systematically poisoned (3).

Determinations of pH values made by the LaMotte colorimetric method indicate that nearly all of the soils of the Ponce area react well above pH 7.0 in the surface and well above 8 in the lower B horizon. The soils of the coastal plain, peat and muck excepted, usually react above pH 8.0 at the surface.

In local spots scattered over the coastal and inner plains the pH has been considerably raised by thick deposits of seashells mixed with wood ashes and broken pottery fragments. These places are the old camping grounds of the aborigines and are known as "kitchen middens." Many very interesting implements have been found in these places.

### *Solonchak soils*

The solonchak soils, or those containing large quantities of salts, may be grouped into two or more classes according to the relative proportions of sodium carbonate to the other soluble salts. In making a map of these soils for the

use of the experiment station, 16 classes were separated. These separations were based on the percentage of total salts as determined in the field by the electrolytic bridge and the approximate ratios of sodium carbonate to other salts as determined by the strength of color produced with phenolphthalein. Obviously the latter test cannot be considered as quantitative. The information obtained is shown in table 2.

Salt crusts were collected from different localities and analyses for the ions indicated in table 3 were made. The following methods of analysis were used in determining the ions:

Na<sup>+</sup>,—zinc uranyl-acetate  
Mg<sup>++</sup> and SO<sup>--</sup>,—gravimetric method  
Remainder by volumetric method

TABLE 2  
*Field tests on 198 4-foot alkali soil samples*

	REACTION WITH PHENOLPHTHALEIN*				
	Red	Pink	Pale Pink	No color	Totals
Reaction with phenolphthalein increases downward, indicating downward proportional increase in Na <sub>2</sub> CO <sub>3</sub> in relation to other salts . . . . .	10	13	35	0	58
Reaction with phenolphthalein decreases downward, indicating conditions opposite to above . . . . .	20	14	13	0	47
Percentage of total salts increases downward . . . . .	0	9	19	23	51
Percentage of total salts decreases downward . . . . .	39	22	40	46	147
Percentage of total salts to dry soil for entire 4-foot profile:					
Less than 0.2 per cent . . . . .	0	1	8	20	29
0 2-0 4 per cent . . . . .	1	6	12	22	41
0 4-0 6 per cent . . . . .	11	14	8	6	39
0 6-1 0 per cent . . . . .	13	6	12	8	39
1 0-3 0 per cent (and over) . . . . .	14	4	19	13	50

\* Numbers indicate the number of 4-foot samples in each category.

Although the results of the analyses of samples II, V, VII, and VIII do not agree well with the totals yet they will serve in a general way to show the approximate proportions of the different water-soluble salts.

Samples I and VI are "white alkali" with sodium chloride predominating and with just a little carbonate and bicarbonate in each. Sample II is an excellent example of a balanced "white alkali" in which all of the common "white alkali" salts are present, but in which sodium carbonate is lacking. In samples III, V, and VII, "white alkali" predominates but there are appreciable quantities of "black alkali." Samples IV and VIII are typical "black alkalis," with large quantities of sodium carbonate present.

There are several sources which contribute to the supply of soluble salts which occur in the solonchak soils of this region. The alluvial fans have been gradually encroaching on the Caribbean Sea and its various lagoons and estuaries. All of these bodies of water are more or less salty or brackish, the principal salt being NaCl. Some of the limy shales, such as those from which soil profile 1 is derived, carry considerable quantities of gypsum. Crystals of this salt were found in a fresh cut which was being made through a hill for the accommodation of an irrigation ditch. In the same place a small flow of water was encountered which contained nearly 2 per cent NaCl. Sodium carbonate and calcium chloride may be produced by the reaction of calcium

TABLE 3  
*Chemical analysis of alkali crusts from salty soils, Ponce Area\**

NO AND LOCATION OF SOIL SAMPLE	I	II	III	IV	V	VI	VII	VIII
	0.4 KM. EAST OF PLAYA ON CENTRAL CONSTANCIA ROAD	0.4 KM. NORTH OF PAS-TILLO ON URSULA ROAD	0.4 KM. EAST OF PAS-TILLO	1.4 KM. SOUTH OF HCDA. ESPERANZA PLAZA	1.2 KM. SOUTH ON ROAD BETWEEN LOS PAMPANOS AND THE PLAYA CEMETRY	JUST WEST OF MERCE-DITA GATE	JUST WEST OF RIO FORTUQUES AT PLAYA PONCE	SALTY SOIL NEAR GATE TO HCDA SERRANO
	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.
Total solids	65,615	17,980	6,548	23,480	14,089	158,379	93,312	7,946
Lost on ignition		3,499	1,198	2,163	2,958			1,564
Calcium	161	824	96	101	87	1,794	3,555	70
Sodium	24,253	3,535	1,632	5,106	5,145	58,680	20,175	2,623
Magnesium	148	477	.....	.....	56 0	2,601	4,190	37 0
Carbonates	51.4	.....	100	3,040	525	76	103	2,400
Bicarbonates	317 0	322	580	2,542	1,293	583	247	1,923
Chlorides	37,639	5,725	1,861	5,096	1,623	65,007	42,106	533 0
Sulfates	1,784	1,491	898	5,106	7,334	24,428	5,174	653

\* Analyses by Mr. Jose H. Ramirez, chemist, Insular Experiment Station.

bicarbonate and sodium chloride (4). Some authors, however, question whether this reaction takes place in the soil (6). This fact was noted in the field at any rate: Excess NaCl and  $\text{Na}_2\text{SO}_4$  are present in the moist soils adjacent to the aforementioned lagoons and estuaries, and  $\text{Na}_2\text{CO}_3$  is of common occurrence where these poorly drained salty soils come into contact with those containing more or less calcium carbonate.

The 198 4-foot alkali samples<sup>3</sup> shown in table 3 bring out the following points of interest:

<sup>3</sup> These samples were taken during the dry season when efflorescences were common on the surfaces of the alkali soils.

Sufficient color with phenolphthalein to indicate the presence of appreciable quantities of  $\text{Na}_2\text{CO}_3$  (or  $\text{K}_2\text{CO}_3$ ) was shown by 65.2 per cent of all samples taken. A medium to strong reaction with phenolphthalein was shown by 30.3 per cent of all samples.

When the ratio of sodium carbonate to other salts is low the former is more concentrated in the subsoil than at the surface.

Vice versa, when the proportion of sodium carbonate is high it tends to concentrate in the surface horizon.

There is a general tendency for all salts to be more concentrated in the surface horizon than in the subsoil.

A study was begun of the principal halophytic plants in relation to the percentage and kinds of salts in the alkali areas. This material is presented in brief form in the following.<sup>4</sup>

Two of the most satisfactory alkali indicators are the verdolaga rosada (*Sesuvium portulacastrum*) and the barilla (*Batis maritima*). The former is most commonly found on soils containing from 0.6 per cent to 3.0 per cent total salts of which only a small proportion is sodium carbonate. It also grows on white alkali soils where the total salts exceed 1.0 per cent. It is occasionally found on soils having a low percentage of salts most of which is sodium carbonate. The barilla is confined almost entirely to areas where the salts are chiefly of the "white alkali" variety (mostly  $\text{NaCl}$ ) and where the percentage is between 0.6 and 3.0.

Sedges (*Fimbristylis*) are practically always on soils which are moderately salty. Their presence is dependent largely on having very moist soil which, a large part of the time, is actually wet. The enneas or cattails (*Typha angustifolia*) grow in marshy places. There is usually less than 0.4 per cent salts where the enneas grow in abundance. Mangles or mangroves of several species grow in marshy soils near the sea and in semi-marshy flats associated with barilla. They are commonest where the ratio of sodium carbonate to other salts is low, but they will flourish in any wet alkali soil where the salt percentages range between 0.2 and 3.0. The matojo de playa (*Sporobolus virginicus*) usually grows in salty soils near the sea. It will tolerate a high percentage of salts but is not common on black alkali soils. *Jaquemontia subsalina* and *Evolvulus glaber* are good salt indicators but are not easily noticeable. They are both small creeping vines with pale blue flowers. They occur on alkali soils where the ratio of sodium carbonate to white alkali is small and where the total salts range from 0.4 per cent to about 2.0 per cent. These last figures are from rather meager data. The cotorrera de playa (*Heliotropium curassavicum*) is fairly common on all grades of alkali land except those where the salt percentage is very high.

One of the commonest plants on the alkali soils of the Ponce area is the grass known as horquetillo (*Chloris radiata*). It grows on all grades of alkali soil

<sup>4</sup> We are indebted to Dr. N. L. Britton of the New York Botanical Gardens for identifications of plants.

but cannot be used as a sure indicator because it also flourishes on non-alkali soils. There are many other plants, such as various cacti, shrubs, and trees, which fall into the same category.

A poor grade of sugar cane was observed growing on soils where the percentage of alkali runs as high as 0.6 per cent. Where the alkali is of the white variety the cane shows little damage under percentages up to 0.4 per cent but noticeable damage from black alkali occurs with 0.2 per cent or less.

#### SUMMARY

Soils discussed in this paper are found in south central Puerto Rico where the climate is warm and comparatively dry. The average annual temperature is 78° and the annual precipitation varies from 25 to 60 inches. As a result of the high annual evaporation the mature soils of the region belong to the chernozem and chestnut-colored soil groups. The normal profiles for these soils are not developed but take the form of chernozem-"clay pans" and chestnut-colored "clay pans"

Where the alluvial fans of the coastal plain approach the marshy lands and lagoons bordering the Caribbean Sea, alkali and soluble salts have accumulated in many of the soils to such an extent as to retard or prevent agricultural pursuits. These "alkali spots" support a typical halophytic vegetation.

All soils of the area, except peat and muck, so far as observed, have pH values well over 7.0, many of them exceeding 8.0. Worms and insects have accomplished much in the translocation of soil materials from one horizon to another in all of the soils except those which are very salty or very wet. In the latter the land crabs are very active in transferring material from the subsoil to the surface.

A list of the principal halophytic plants and the types of salty lands to which they are adapted is given.

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## PLATE 1

## SOILS OF PUERTO RICO

FIG. 1. Note the coarsely granular surface soil, the exceedingly heavy and cloddy upper subsoil, and the white silty and concretionary line accumulations.

FIG. 2. A chernozem-clay pan soil just west of Coamo Springs. Note the white, soft lime concretions above and silty lime below; also the prismatic structure to the left of the pick. Surface not shown.

FIG. 3. Hard limestone hill southwest of Coamo Springs. Soils are rendzinas. River terrace in foreground.



FIG. 1



FIG. 2

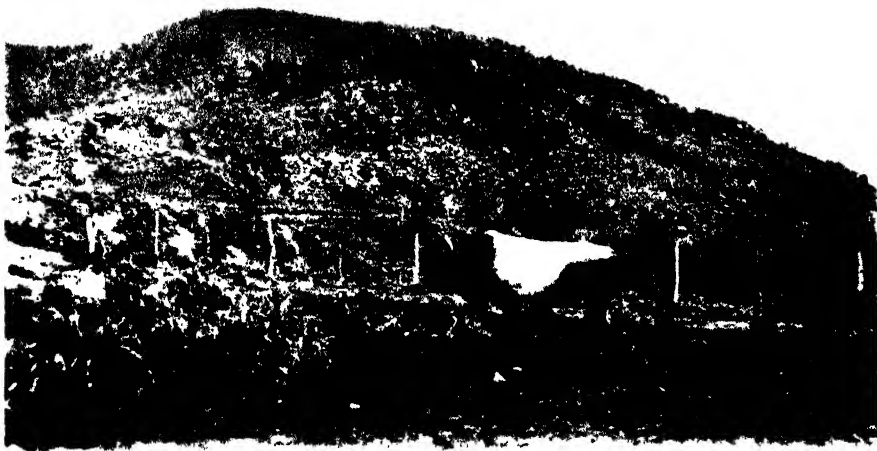


FIG. 3



## PLATE 2

## VEGETATION ON PUERTO RICO SOILS

FIG. 1. Heavy sugar cane yield on recent alluvial soils near Hacienda Cortada. This field produced 75 tons an acre in the spring of 1930. *Gran cultura* crop.

FIG. 2. A study of halophytic vegetation near the south coast of Puerto Rico. Mangroves in left background. Colony of barilla in foreground. *Verdolaga rosada* (*Sesuvium*) just to left of figure. Matojo de playa between figure and right background. White patches are crystallized soluble salts.



FIG. 1



FIG. 2



# A METHOD FOR DETERMINING COMBINED WATER AND ORGANIC MATTER IN SOILS<sup>1</sup>

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Combined water in soils presents a problem of the most important and fundamental nature because of the rôle it plays in their mechanical and chemical analyses and in the determination of their organic matter. By combined water, is meant that water which remains in soils after they have been dried at a temperature of 108°C. for 24 hours. It may be water of crystallization, constitution, or imbibition, or combinations of these. Although its existence in soils and its importance are generally recognized, there is at present no method for its direct determination.

In the course of study of soil moisture at this laboratory (1-6), the idea occurred to the writer that the combined water in soils might be directly determined by means of distillation at a high temperature. It seemed possible that if this method succeeded, then the ignition method for determining organic matter would become more accurate and dependable, since the great source of error in this method is the uncertainty concerning the quantity of combined water present in soils.

Happily, the idea and principle of determining the combined water by the distillation method seem to be sound and successful and, because of this success, the ignition method for determining organic matter in soils becomes more accurate and reliable. In addition, the distillation method can also be used to determine the total water content in soils. This latter subject, however, will be considered in a subsequent paper. Although the method seems to have proved successful and has given definite results, yet this report should be considered as preliminary to a more complete investigation. The investigation of the whole subject is being continued with special effort to perfect further the technique and apparatus.

## METHOD AND PROCEDURE

The distillation apparatus employed for determining the combined water in soils is shown in plate 1. It consists of a 250-cc. bomb made of iron pipe. The cap is screwed on. In a hole in the center of the cap is brazed a short

<sup>1</sup> Journal Article No. 103 (U. S.) from the Michigan Agricultural Experiment Station.

<sup>2</sup> The chronological order of publication of this paper and that of one by the same author, dated March 21, are reversed at the author's request.

$\frac{1}{8}$ -inch pipe and to this pipe is brazed a copper tube  $\frac{1}{8}$  inch inside diameter and 2 feet long. This copper tube is passed through a condenser made of lead pipe soldered near the center of the copper tube. The condenser can be either connected to a faucet with running water or kept filled with cold water. The combined water is caught in a special very narrow cylinder graduated to 0.1 cc. and containing carbon tetrachloride. The carbon tetrachloride is essential because it dissolves or coagulates any organic matter that may be distilled over and greatly facilitates the accuracy of reading the water column in the cylinder.

The procedure for determining the combined water consists of first preparing the soil by passing it through a 2-mm. sieve then testing it for carbonates. If carbonates are present they are destroyed with hydrochloric acid and then the soil is thoroughly washed, dried, and screened again. Soils that have no carbonates are not treated with the acid. Exactly 100 gm. of the air-dry soil is then placed in the bomb and about 30 gm. in a crucible. The latter sample is used to determine the hygroscopic moisture and the ignition loss of the soil.

TABLE 1  
*Tests of the degree of accuracy of the distillation method*

SOILS	WATER ADDED	WATER RECOVERED
	cc	cc
Quartz sand, dried	10 00	10 00
Davidson loam, ignited	10 00	9.90
Ontonogan clay, ignited	10 00	10 00
Fargo clay loam, ignited	10 00	9 90
McKenzie clay, ignited	10 00	9 90

The lid is screwed on the bomb and the joints are covered with a layer of asbestos cement to make sure that the bomb is air-tight. The process of putting on and taking off this asbestos cement is very simple and does not take more than two minutes. The bomb is now placed in the electric muffle, the cylinder containing the carbon tetrachloride is connected to the end of the copper tube, and the condenser is connected to the faucet. The current is turned on and regulated to produce a temperature of 800°C. in the case of mineral soils and about 330°C. in the case of peats and mucks. The water distills very rapidly and rises to the top of the carbon tetrachloride. It is very clear except in peats, in mucks, and in mineral soils containing an extremely large amount of organic matter, in which cases it is slightly brown. There may also be a thin layer of coagulated organic matter between the water and carbon tetrachloride in case of some of the organic soils but the error that may arise from it is negligible. The water standing above the column of carbon tetrachloride represents both the hygroscopic and combined water of the soil and its volume is read and recorded. When distillation is complete the bomb is replaced in the muffle by another one containing a different soil. By having a

set of three or four bombs, which can be easily made at a machine shop for about \$2 each, and by maintaining the temperature of the muffle at 800°C., the combined water of three or four soils can be determined in about one hour. It was experimentally found that if a soil is placed in a furnace at a temperature of 800°C. no more water is distilled from it after 15 minutes.

Before the method was employed to obtain final experimental results its degree of accuracy was tested by adding a definite amount of water to dried quartz sand and to ignited soils having no hygroscopic or combined water, and then recovering the water added. Before this was done, however, the attraction of the copper tube for a film of water was satisfied by running one or two preliminary experiments. The results obtained are shown in table 1.

It is seen that the quantity of water recovered in each case agrees quite satisfactorily with the quantity added.

The two great sources of error in the method lie in not having the cap on the bomb air-tight and in the danger of some soil getting into the copper tube and thus either preventing the distillation or absorbing some of the water that is being distilled. The first danger is completely eliminated by covering the joint between the cap and the bomb with asbestos cement. The second danger can be easily avoided by care in handling the bomb after the soil is placed in it. The copper tube should also be frequently cleaned with alcohol and water. It is advisable to use a separate bomb for the organic soils.

#### EXPERIMENT RESULTS

In table 2 are presented the experimental results as obtained by the distillation method on the combined water and organic matter in soils. The combined water is the difference between the hygroscopic water lost at the temperature of 108°C. for 24 hours and the total water distilled from the air-dry soil. The ignition loss was obtained in the electric muffler at the temperature of 800°C.—the same as that used for distillation—on soils previously dried at 108°C. for 24 hours. The organic matter is obtained by subtracting the combined water from the total ignition loss. No correction is made for carbonates, since they were destroyed by the acid treatment.

The results in table 2 show that the combined water varies greatly in the different soils.

The results on combined water and ignition loss of the first 11 soils present the real test as to the soundness, accuracy, and reliability of the distillation method for determining combined water in soils. These first 11 soils and artificial materials, as far as could be ascertained, contain no organic matter or carbonates. Provided that the loss on ignition is due to combined water alone, then the combined water as obtained by the distillation method should equal the loss on ignition. That is exactly what takes place. It will be seen that the figures for combined water and ignition loss for all of these 11 soils and artificial materials, are remarkably close. This close agreement is significant and should be considered as affording a real test for the method.

When the soils containing organic matter are examined, however, it is seen that the figures for combined water and ignition loss are far from being in close

TABLE 2

*Combined water as obtained by the distillation method, and organic matter determined indirectly by calculating the difference between combined water and ignition loss*

Soils 1 to 11 contained no organic matter or carbonates

SOILS	CaCO <sub>3</sub>	HYGRO- SCOPIC MOISTURE AT 108°C.	LOSS ON IGNITION FROM SOILS DRIFT AT 108°C.	COMBINED WATER DISTILLED OVER FROM SOILS DRIED AT 108°C.	ORGANIC MATTER CORRECTED FOR CARBONATES AND COMBINED WATER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. Silica gel . . . . .	0	3.79	2.92	3.02	
2. Bentonite . . . . .	0	5.64	2.56	2.59	
3. Susquehanna clay, 16-20 inches. . .	0	2.73	6.78	6.68	
4. Davidson clay loam, 24-30 inches . .	0	1.71	8.18	8.20	
5. Nype clay . . . . .	0	2.48	10.67	11.22	
6. Parson silt loam, B horizon . . . . .	0	2.16	6.06	6.88	
7. Decatur clay, 6-12 inches . . . . .	0	1.48	4.78	4.68	
8. Iredell loam, 12-16 inches. . . . .	0	4.18	8.70	9.42	
9. Bladen loam, 12-8 inches . . . . .	0	1.87	5.25	5.66	
10. Antioch clay loam, 40-60 inches. . .	0	2.97	2.92	2.86	
11. Greenville fine sandy loam 12-18 inches . . . . .	0	1.20	4.74	4.18	
12. Capay clay . . . . .	2.20	4.12	4.47	4.22	
13. Black clay adobe . . . . .	0	6.06	8.30	5.84	2.46
14. McKenzie clay . . . . .	0	4.40	10.00	4.65	5.35
15. Clarion silt loam, surface . . . . .	0	2.59	6.18	2.67	3.51
16. Houston black clay, 1-12 inches . .	24.90	2.20	8.33	3.63	4.70
17. Fargo clay, surface . . . . .	0	5.90	11.70	5.90	5.80
18. Fulton clay, subsoil . . . . .	15.80	2.02	4.50	3.98	
19. Haldemond clay, subsoil . . . . .	0	2.85	8.91	3.99	4.92
20. Ontonagon clay, C horizon . . . . .	28.40	1.43	3.01	2.31	
21. Lake Charles clay, surface . . . . .	0	3.43	5.42	2.95	2.47
22. Clyde clay, surface . . . . .	0	4.95	17.30	8.40	8.50
23. Lindly sandy loam, surface. . . . .	0	1.57	4.82	2.38	2.44
24. Davidson loam, surface . . . . .	0	1.72	9.60	5.58	4.02
25. Clinton silt loam, surface . . . . .	0	1.43	5.10	1.97	3.13
26. Putnam silt loam, surface . . . . .	0	1.88	6.33	2.40	3.93
27. Barnes loam, subsoil . . . . .	15.90	2.04	3.20	2.98	
28. Muck 1 . . . . .	0	18.20	79.80	9.5	
29. Muck 2 . . . . .	0	10.00	73.20	10.4	
30. Raw peat . . . . .	0	18.10	94.2	11.0	

agreement. This disagreement is, of course, due mainly, if not wholly, to organic matter, which is present in considerable amounts in some soils, as revealed in the last column of the table.

Since the differences between the ignition loss and combined water amount to about 0.5 per cent in soils that are not supposed to contain organic matter, any differences much less than 1 per cent have probably no significance as far as organic matter content is concerned.

It was feared at the outset that the distillation method might fail in peats and mucks and in mineral soils containing much organic matter because of the possibility that in the destructive distillation of organic matter some water might be formed. Though it is a fact that some plant materials, such as wheat flour, will form water on destructive distillation, no positive evidence is indicated in the aforementioned experimental results that the peats, mucks, and mineral soils with organic matter form water on destructive distillation. This conclusion is based principally on the comparatively small amount of combined water obtained from these organic materials. For instance, in muck 1, muck 2, and in the peat, the percentages of combined water are 9.5, 10.4, and 11.0, respectively, and in Clyde clay, which contains 8.5 per cent organic matter, it is 8.40 per cent. In contrast, the combined water in Davidson loam, Nype clay, and Iredell loam, which contain no organic matter, is 8.20, 11.22, and 9.42 per cent respectively. Even if there is a tendency for organic matter to form water upon destructive distillation, the amount would be very small in mineral soils considering their small content of organic matter.

It has already been stated that the temperature used for distilling combined water from peats and mucks is about 330°C., whereas for the mineral soils it is 800°C. The lower temperature for peats and mucks is used because at the higher temperature so much organic matter is distilled over that it is very difficult to measure accurately the combined water. At the temperature of 330°C. very little, if any, organic matter is distilled over, and the water distilled is practically clear and can be measured very accurately.

The point may be raised that if a temperature of 800°C. is required to distill the combined water from the mineral soils, then the temperature of 330°C. may not be sufficiently high to distil all of the combined water from the peats and mucks. It must be stated that even when the higher temperature was used on the peats and mucks it seemed that no more combined water was obtained than at the lower temperature. In fact, the impression has been gained from the various experiments performed that organic matter gives up its combined water with comparative ease and does not require anywhere near as high a temperature as some of the mineral constituents to free it of combined water. Furthermore, it does not seem reasonable that organic matter will fail to give up its combined water at the temperature of 330°C. when it itself begins to break down and distil over at this temperature.

#### *Relationship between combined water and clay content of soils*

A comparison of the relationship between the combined water and clay content of soils is shown in table 3. It is evident from this comparison that



though there is a general tendency for the combined water to vary with the clay content of soils, yet there are several soils in which the relationship is not at all close. This is probably as should be expected because not only clay content but also chemical composition should affect the amount of combined water.

TABLE 3  
*Comparison between combined water and clay content of soils*

	COMBINED WATER	CLAY (.005-0 mm)	FINE CLAY (.002-0 mm.)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Susquehanna clay, 10-20 inches . . .	6 68	41 0	39.5
Davidson clay loam, 24-30 inches . . .	8.20	71 2	69 1
Nype clay . . . . .	11.22		
Parsons silt loam, B horizon. . . . .	6.88	64 3	60.1
Decatur clay, 6-12 inches . . . . .	4 68	42 3	40 7
Iredell loam, 12-16 inches . . . . .	9 42	74 1	70 4
Bladen loam, 12-18 inches . . . . .	5 66	54 0	48 8
Capay clay adobe . . . . .	4 22	63 2	56 9
Antinoh clay loam, 40-60 inches . . .	2.86	34 8	32 4
Greenville fine sandy loam, 12-18 inches . .	4 18	39 6	37 5
Black clay adobe . . . . .	5 84	55 9	52 7
McKenzie clay . . . . .	4 65	76 1	71.9
Clarion silt loam, surface . . . . .	2 67	24 3	21 8
Houston black clay, 1-12 inches . . . . .	3 60	57 2	52 8
Fargo clay, surface . . . . .	5 90	60 4	55 1
Fulton clay, subsoil . . . . .	3 98	68 0	66 5
Holademonid clay, surface . . . . .	3 99	85 6	77 4
Ontonagon clay, C horizon . . . . .	2 31	79 8	72.7
Lake Charles clay, surface . . . . .	2 95	42 4	38 9
Clyde clay, surface . . . . .	8 40	51 7	48 6
Lindly sandy loam, surface . . . . .	2 38	29 2	23.4
Davidson loam, surface . . . . .	5 58	43 3	36.7
Clinton silt loam, surface . . . . .	1 97	28.2	23 4
Putnam silt loam, surface . . . . .	2 40	24 2	20 5
Barnes loam, subsoil . . . . .	2 98	32 2	29 2

#### SUMMARY

A successful method of distillation is presented for determining the combined water in soils.

The soil is placed in a bomb and heated at a high temperature. The combined water is distilled over very rapidly, condensed, and caught in carbon tetrachloride.

Because the combined water can be determined, the ignition method now becomes more accurate and reliable for determining the organic matter in soils.

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## PLATE 1

BOMB USED FOR DETERMINING COMBINED WATER BY DISTILLATION





# ON THE DETERMINATION OF THE ION EXCHANGE CAPACITY OF SOILS

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Many investigations show that the replacement of ions in soils for the purpose of determining their exchangeable quantities presents much difficulty. As it recently was pointed out by Gedroiz (4), one fraction of the cations in soils may be easily replaced by some other, but there exists another part which can only be replaced with considerable difficulty, and the reaction never reaches completion within a reasonable time. Gedroiz, therefore, makes a distinction between *intensively* and *extensively* exchangeable bases in soils. Several works of Goy (5) show interesting data along these lines on the exchangeable H ions of soils. On the above basis the author drew a distinction between "easily soluble acid" and "difficultly soluble acid" in soil ("leichtlösliche Säure" and "schwerlösliche Säure"). Kappen drew a similar distinction between "exchange acidity" and "hydrolytic acidity" several years ago.

There is, however, no justification for such arbitrary distinctions. The ion exchange reactions in soils and in other colloidal materials capable of ion exchange seem to follow the laws valid in the case of any reactions influenced by different degrees of dissociation of the reacting ions, as has been shown in several papers by Mattson (9). Results published by the author some time ago (1) also showed that, in respect to the reagent used to effect the H ion exchange, the higher its ratio to the amount of soil taken, the higher the amount of exchanged H ions, and in the case of each soil the graphical representation of the relation between the amounts of exchanged ions and the quantity of replacing reagent used, is a very regular adsorption curve. Vageler and Woltersdorf (13) in a theoretical discussion of these experimental results and of the results of other workers have shown that the curve is a hyperbola corresponding to the hyperboloid form of the dissociation residue curve, and can be characterized by the asymptote formula of the hyperbola as follows:

$$\frac{x \cdot S}{x + C}$$

where  $x$  = the quantity of reagent used for ion exchange, expressed in milligram equivalents;

<sup>1</sup> At present, research assistant at the New Jersey Agricultural Experiment Station.

$y$  = the exchanged ions found, expressed in milligram equivalents;

$S$  = the limiting value of the adsorption capacity with respect to the replacing ion;

$C$  = the half value of the limiting value.

If, instead of using the actual values, the reciprocal values are used, the graph becomes a straight line, which can be represented by the following formula:

$$b = K + q a$$

$$\text{Where } b = \frac{1,000}{y}; K = \frac{1,000}{S}; a = \frac{1,000}{x}; q = \frac{C}{S}.$$

The  $q$  value—the tangent of the angle of inclination—can be calculated from two determinations, which give two points on the hyperbola (i.e. on the straight line, if the reciprocal values are taken), as follows:

$$q = \frac{b_1 - b_2}{a_1 - a_2}$$

It therefore follows that *no absolute value* can be obtained for the end of the ion exchange reaction at any finite dilution, but Vageler and Woltersdorf consider that *comparable values for ion exchange capacity of different complexes are represented by the limiting values* calculated on the foregoing basis (13, 14). In a subsequent publication (12) Vageler points out that the  $q$  value "probably represents the reciprocal of a value which seems to be proportional with the dissociation constant of the colloid."

A. Bernolak and the author have obtained further data along this line on eight unsaturated soils obtained from areas which differed widely in all soil characteristics.

A description and some characteristic data for these soils are given in table 1.

These data show that these soils differ widely in their properties.

In order to determine the  $q$  value with respect to the exchangeable H ions of these soils, the same amount of soil was treated with two different quantities of a normal calcium acetate solution and the mixtures were allowed to come to equilibrium, then filtered, and the increase of H ions in the filtrate was determined by titration. The  $q$  values were calculated by substitution in the formula. The data are given in table 2. In figure 1 the  $q$  values are plotted against the quantity of exchanged H ion found with a 1:2.5 ratio of soil to reagent. (The quantity of soil is expressed in grams on 105°C. oven-dry basis, the quantity of reagent in milligram equivalents of a normal solution, respectively.)

From this curve the  $q$  value appears to be intimately correlated with the H ions exchanged at a certain ratio, irrespective of the nature of the soil and seems to be, therefore, a function of the degree of dissociation of the acidoid alone, i.e., of the activity of exchangeable H ions present in the complex. In other words: *those quantities of soils or other materials capable of ion exchange, which*

are equivalent with respect to their ion exchange capacities must show the same ion exchange, if treated with the same amount of replacing reagent.

TABLE 1  
General data\* on the soils used in the experiments

NUMBER	DESCRIPTION OF THE SOILS	pH (IN WATER SUS- PENSION)	EXCHANGE- ABLE CATIONS (S ACCORD- ING TO HISSINK)	(T-S) (EXCHANGE- ABLE H <sup>+</sup> )	T (CATION- EXCHANGE CAPACITY)	V 100 S/T
			m.e. per 100 gm. soil 1:105°C. oven-dry			
						<i>per cent</i>
59	Sand, very low in organic matter	7 02	2 04	2 50	4 54	44.9
51	Sand, very low in organic matter	6 56	1 80	2 58	4 38	41.1
88	Sand, low in organic matter	6 11	2 88	4 03	6 91	41.7
190	Clay, low in organic matter	4 87	20 80	11 21	32 01	65.0
431	Clay, low in organic matter	5 89	37 38	12 45	49 83	75 0
16	Loam, low in organic matter	6 01	19 40	6 57	25 97	74.7
1198	Clay loam, medium in organic matter	6 05	30 50	17 23	47 73	63 9
1404	Loam, rich in organic matter	6 85	47 36	14 06	52 02	91 0

\* The exchangeable bases have been obtained by electrodialysis; the (T-S) values are calculated on the basis of the hydrolytic acidity according to Kappen (7). T and V values, according to Hissink (6), have been calculated on the basis of the values obtained by the above determinations.

TABLE 2  
Exchanged H<sup>+</sup> at different soil reagent ratios

NUMBER	EXCHANGED H <sup>+</sup> M E PER 100 gm. 105°C OVEN-DRY SOIL		q
	1 2 5 ratio, gm m e soil: reagent	1:25 ratio, gm soil: m e. reagent	
59	0 77	2 71	258.3
51	0 79	2 86	254.5
88	1.24	2 91	128.6
190	3 45	8 50	47.9
431	3 83	8 67	40.5
16	2 02	5 87	90.2
1198	5 30	10.00	24.6
1404	1 25	3 00	129.6



Results obtained for the ion exchange capacity by any method can be checked up on this basis by weighing out quantities of the materials, which, according to the results obtained by the method in question, should be equivalent with respect to their ion exchange capacities. If the method gave the correct, i.e., comparable, results, equal amounts of exchanged ions must be found, if equivalent weights of soils have been treated with equal amounts of reagent.

We carried out experiments with our soils along this line. A method for the determination of the exchangeable H ions in soils suggested by the author (1) was used. Quantities of soils which were equivalent with respect to their exchangeable H ions were calculated and weighed out according to the results obtained by this method, and treated in one series with 250 cc. and in another

TABLE 3  
*Comparison of results of exchangeable  $H^+$  on equivalent quantities of soils*

NUMBER	EXCHANGEABLE $H^+$ M.E. PER 100 GM 105°C. OVEN-DRY SOIL ACCORDING TO THE AUTHOR'S METHOD	EQUIVALENT WEIGHTS (QUANTITY OF SOIL CORRESPONDING TO 1.88 M.E. OF EXCHANGEABLE $H^+$ )	EXCHANGED $H^+$ IN M.E. PER EQUIVALENT WEIGHTS	
			250 cc. of replacing reagents per equivalent weight	250 cc. of replacing reagents per equivalent weight
		grams		
59	2 63	71 48	0 69	1 70
51	1 88	100 00	0 70	1 70
88	2 38	78 99	0 69	1 72
190	20 50	9 17	0 89	2 50
431	13 50	13 93	0 89	2 50
16	10 50	17 90	0 88	2 50
1198	27 50	6 84	0 89	2 50
1404	7 50	25 07	0 88	2 50

with 2,500 cc. of a normal calcium acetate solution. The data of this experiment are shown in table 3. The results show extremely good agreement, the soils, however, fall into two groups: (a) Sandy soils very low in exchangeable H ions, which give a good agreement amongst themselves, give uniformly lower results, if compared with the other group; (b) loam and clay soils high in exchangeable H ions also show good agreement among themselves but give higher results than the soils of the former group.

Subsequent experimental work carried out by the author at the New Jersey Agricultural Experiment Station has shown that this difference arises from the fact that a comparatively large amount of sandy soil has to be taken to give equivalents of exchangeable H ions with the loam and clay soils. With the loam and clay equivalent weights the results are obtained therefore from considerably lower soil/reagent ratios than with the sand equivalent weights, and the results on such a basis are not strictly comparable, as was shown by the following experiment:—Twelve very different electrodyalyzed soils and other

colloidal materials were used and the displacement of H ions by Ba ions from a normal barium acetate solution was determined at three different ratios. Then the  $q$  values were calculated, first from the two higher, and afterwards from the two lower ratios. These values were plotted against the amounts of H ions exchanged at the higher ratios, in figure 2, in the same way as in the figure 1. The graph shows very well that the same results cannot be obtained, if the values are calculated from results obtained at extremely different ratios,

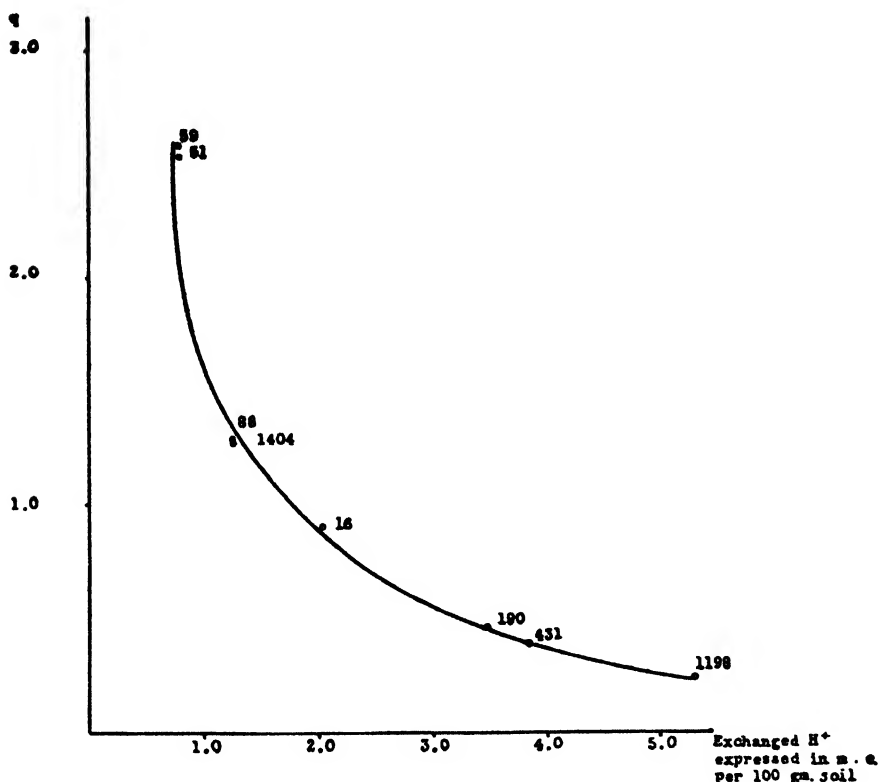


FIG. 1. RELATIONSHIP BETWEEN H<sup>+</sup> EXCHANGED 1:2.5 SOIL/REAGENT RATIO AND THE  $q$  VALUE (EXPERIMENTAL SOILS)

and that the shifting of the results is *different* in the case of materials with low and high ion exchange capacities. The lower the ion exchange capacity, the higher the increase of the  $q$  values, if calculated from lower and lower ratios. Hence, the difference obtained with the (a) and (b) groups of soils in the case of the experiment described in the foregoing. If we look at the curves of figure 2, and consider their relationship with the experiment described, we find that the results obtained with the loam and clay soils must lie on curves corresponding with the B curve of figure 2 (low soil/reagent ratio), whereas the results of the sandy soils must lie on curves corresponding with the A curve

(high soil/reagent ratio). Therefore the ion exchange of equivalent quantities must have been lower in the case of the soils of the (a) group than in the case of the soils of the (b) group.

It still must be pointed out that the relation between the  $q$  value and the ion exchange at a certain ratio does not agree as well, if the comparison is made on the basis of results obtained by very diluted suspensions (low soil/reagent ratios). The  $q$  values calculated by the results of the 1:20 and 1:100 dilutions give considerable irregularities with respect to this relationship, as is shown by

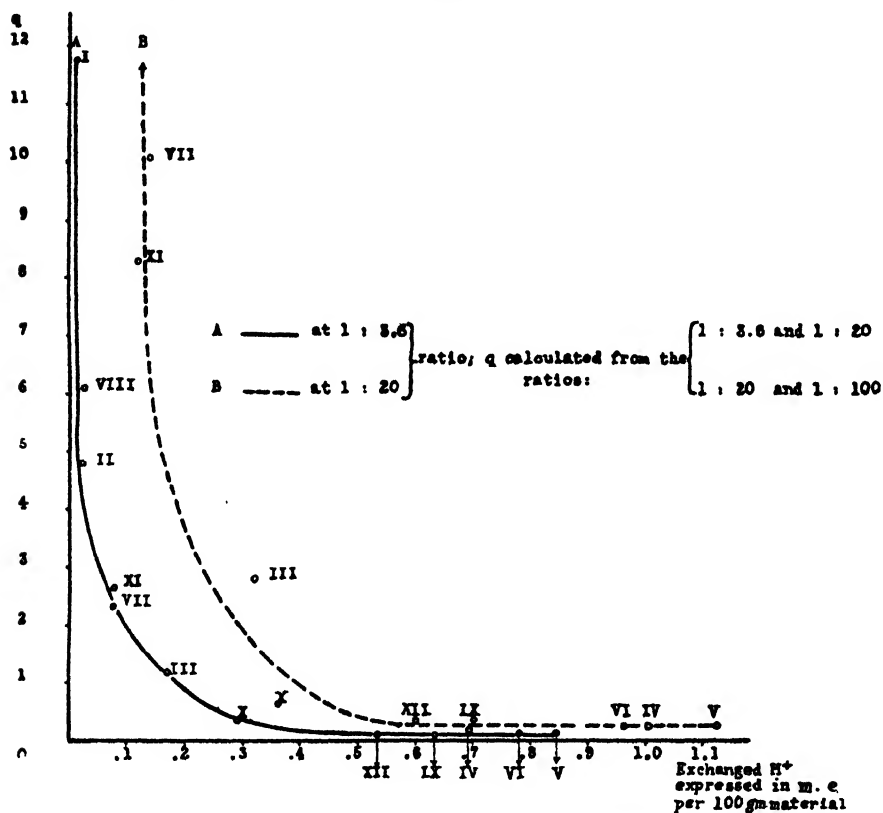


FIG. 2. RELATIONSHIP BETWEEN  $H^+$  EXCHANGED AT DIFFERENT MATERIAL/REAGENT RATIOS AND THE  $q$  VALUE (SOILS AND OTHER COLLOIDS)

curve B in figure 2. It seems that the concentration of the suspension itself influences the results to a comparatively small extent, and this influence may, in all probability, vary according to the nature of the physical properties of the material. Besides this, the adsorption curve may still be affected by other factors, namely, by reactions and dissociation phenomena of the other ions present; for example, by anion exchange.

Nevertheless we can see that the influence of the exchangeable amount of the investigated ion present, i.e., its power to dissociate, suppresses almost all

the influences of this kind, at least at high material/reagent ratios. If so, we have the possibility of estimating equivalent amounts of different materials with respect to their exchangeable ion by interpolation, i.e., by calculation with the formula of the adsorption curve. Equal amounts of exchanged ions must belong to equivalent quantities, if the same amounts of replacing reagent are used, as this was shown. Hence, in order to obtain equal amounts of exchanged ions, different quantities of reagent must be taken. *The quantities of reagent used to obtain equal base exchange must be proportional to the equivalent weights.* The equivalent weights cannot be determined on this basis experimentally because of the already described shifting of the curves, unless the factors affecting this shifting and their quantitative influences are not determined. The calculation of any points on the adsorption curve, however, must give correct results for the purpose of comparison of ion exchange at any ratios if the fundamental requirement is fulfilled, namely, that the  $q$  values calculated from the points determined show a regular decrease in proportion with an increase of the amounts of exchanged ions of the different materials at a certain material/reagent ratio.

The agreement of the results with our experimental soils can be considered to be only approximate from this point of view; probably because the effects described in the foregoing influenced the results to some extent in the case of these very different soils and with the applied ratios. Yet, a comparison of the equivalent weights calculated on the basis of the adsorption curves with the experimental results obtained by independent methods show fairly good agreement even in this case. The data for this comparison have been obtained by a method described in the following.

The exchangeable H ions of the soils were determined by several other methods, which have been recently used for this purpose. The results obtained are given in table 4 and show that a good parallelism of the results was obtained with all of those methods which are based on the determination of replacement of H ions by the metallic cation until a certain pH value is reached (methods of Jensen, Goy, and the author). All the other methods which are based on a direct comparison of the amounts of exchangeable H ions on the basis of the results of a determination of the exchanged H ions at a certain prescribed soil/reagent ratio gave results which often show very considerable discrepancies in comparison with the aforementioned methods. It is also obvious from this that these methods are based on an erroneous assumption, since no comparable results can be obtained if only one arbitrary point of the adsorption curve is taken. Also, by the calculation of the limiting values erroneous results were obtained. This is natural, since this value can only be regarded as representing the *comparative* maximum replaceability of H ions by the cations of the particular solution being used. The end of the reaction could be found only if an infinitely large quantity of reagent were used, and, therefore, equal pH values with the different materials can never be reached at any finite dilution. Therefore, *finite values* calculated on this basis must be erroneous from the point of view of

*comparison of the exchangeable ion* present in the different complexes. This has already been pointed out and shown experimentally in an earlier publication by the author (3).

Now there have been calculated the amounts of reagent which are necessary to obtain equal amounts of exchanged H ions with the different complexes on the basis of the adsorption curve by the formula suggested by Vageler and Woltersdorf. On the basis of two points of the curve given in table 2,  $a$ ,  $b$ ,  $q$ , and  $k$  values were calculated; then  $a_s$  and  $x_s$  values were calculated, taring  $b_1 = 500$ ,  $\gamma_1 = 2.0$ , respectively, in all of the cases. On the basis of  $x_s$  values (i.e., the amounts of reagent necessary to obtain 2.0 m.e. of exchanged H ions

TABLE 4  
*Comparison of the results obtained by different methods for the exchangeable  $H^+$*

NUMBER	EXCHANGEABLE H <sup>+</sup> IN M.E. PER 100 GM. 105°C. OVEN-DRY SOIL								
	Hissink 0.55 T-S (about pH 8)	Hutchin- son- McLennan (about pH 7)	Kappen		Vageler and Woltersdorf limiting value (about pH 8)	Jensen		Goy (pH 7 in N K Cl suspension)	The author (pH 8)
			About pH 7	About pH 8.5		pH 7	pH 8		
						In water suspension			
59	1 39	0 60	1 15	2 50	3 77	0 50	2 75	2 00	2 63
51	1 12	0 80	1 19	2 58	4 03	0 25	2 00	1 75	1 88
88	2 88	1 00	1 86	4 03	3 42	1 50	2 90	2 75	2 38
190	19 79	3 50	5 18	11.21	10 20	10 00	20 0	20 00	20 50
431	11 50	3 80	5.75	12 45	10 10	7 50	15 50	14 50	13 50
16	6 51	2 20	3 03	6 57	7 46	5 00	10 00	9 50	10 50
1198	14.53	4 60	7 95	17 23	11 11	10 00	25 00	25 0	27 50
1404	0 00	1 12	1 88	4 06	3 55	2.25	7 00	7 00	7.50

in all cases) the equivalent weights were calculated, soil 51 being taken as the basis, as follows:

$$\text{Equivalent weight} = \frac{100 \cdot x_3}{x_3 \text{ with soil 51}}$$

The results obtained by this calculation and the equivalent weights calculated from the results obtained by Kappen's method, by the limiting values, and by the author's method are given in table 5.

A rather good agreement between the results obtained by calculation from the curves and those based on the author's method can be found, and although this is only rough, there being even wide discrepancies in two cases (soil 1404 and 59), this comparison seems to strengthen the foregoing statements, as the discrepancies are most likely due to the aforementioned disagreements of the points on the adsorption curves. No correlation can be found in the case of the equivalent weights calculated on the basis of the limiting values, but some interesting confirmations between the interpolated values and the results obtained by Kappen's method can be observed. This fact has a natural

explanation, as these results represent values on the steep part of the adsorption curve where the varying amounts of the exchanged ion often are almost proportional with the ion exchange capacities. The incomparability of the results only becomes more marked when the materials vary greatly in their exchangeable H ions, as is well illustrated by the data in table 5. This is the reason why the unsaturation and lime requirement of soils often can be well characterized for practical purposes by the simple determination of the "hydrolytic acidity" according to Kappen (2, 11). The limiting value does not have this advantage, of course. It shows entirely erroneous values from the point of view of the determination of the amounts of metallic cation necessary to replace H ions until equal pH values are reached, i.e., from the point of view of lime requirement determination. The explanation of this fact has been offered in the foregoing, and is further substantiated by the present data.

TABLE 5

*Comparison of the equivalent weights of the soils with respect to their exchangeable H<sup>+</sup> calculated by the different determinations*

NUMBER	OBTAINED BY INTERPOLATION FROM THE ADSORPTION CURVE	AUTHOR'S METHOD	LIMITING-VALUE OF THE ADSORPTION CURVE (VAGELER'S METHOD)	KAPPEN'S METHOD
59	108 8	71 48	106 89	103 20
51	100 0	100 0	100 00	100 00
88	58 2	78 99	117 8	64 01
190	11 8	9 17	39 51	23 01
431	10 0	13 93	39 90	20 72
16	24 4	17 90	54 02	39 27
1198	5 9	6 84	36 27	14 97
1404	58 9	25 06	113 14	63 55

It must be clarified by further experiments, whether or not we shall be able to obtain adsorption curves due only to the dissociation phenomena of the investigated ions, i.e., independent of physical effects or other ion reactions—since these would enable us to determine the comparative ion exchange capacities of any complex exactly and in the simplest way and to establish accurate comparative values for the dissociation constant of colloidal ionogens.

According to the foregoing, even those methods for the determination of the base exchange capacity of soils which are based on the ideas of Kelley (8) may not give perfect results, unless the replacement of the adsorbed cations is effected by a replacing reagent with well-established pH, the treatment being continued until the original pH value of the reagent is reached. By the use of a large excess of replacing reagent and by other supplementary treatments designed to bring the base exchange reaction to completion, useful results can be obtained, as the error is eliminated to a considerable extent, but the reaction never can be brought to a real completion. The determination of the displaced cations from the material will, therefore, always be susceptible to more or less

error. This error may some time be considerable. Kelley himself observed and pointed out that it may happen that the replacement cannot be brought to completion (8). Besides, a long treatment and leaching are not desirable at all and cannot be applied in some cases, because they may easily affect considerable changes in the structure of the complex, as has been pointed out by Mattson (10).

#### SUMMARY

It was shown that the quantities of exchanged ions of soils treated with increasing amounts of replacing reagent are almost independent of specific soil properties and are influenced by the present amount of exchangeable ion, i.e., by its degree of dissociation only.

The cation adsorption of soils, if increasing amounts of a replacing reagent (calcium acetate solution) are used, gives, therefore, curves which are hyperboloid and can be characterized according to Vageler and Woltersdorf by their asymptote formulas, and different important values can be calculated from two or more points determined on the curve experimentally.

The comparison of values calculated on this basis with independent determinations of the amounts of metallic cation necessary for the displacement of H ions until equal pH values are reached, shows that the equivalent weights of the different complexes with respect to their exchangeable H ions are proportional to the calculated ratios of soil to reagent necessary to effect the same amount of cation exchange.

It seems to be probable that the comparability of the adsorption curves suffered to some small extent because of an influence of the physical properties of the different materials and because of other ionic reactions. A further experimental study along this line is necessary to show whether or not quantitative determinations of exchangeable ions and comparative determinations on ionization of colloidal ionogens can be made on this basis.

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# SOME TRANSFORMATIONS OF UREA AND THEIR RESULTANT EFFECTS ON THE SOIL<sup>1</sup>

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Urea was first discovered in urine by Ronelle in 1773, and was first made from isocyanate of ammonia by Wöhler (21) in 1828. In recent years urea has been synthesized on a commercial scale from calcium cyanamid by various processes which have been patented. Kras, Gaddy, and Clark (11) have also developed a process for its synthesis directly from liquid ammonia and carbon dioxide.

The various processes for the synthesis of urea on a commercial scale have probably been the outgrowth of the rapidly increasing interest in concentrated fertilizers, and the realization that urea would probably be one of the more important nitrogen carriers of the future.

Urea is a compound which analyzes 46.5 per cent nitrogen and is the most concentrated form of nitrogen used as a fertilizer at the present time.

The value of a fertilizer material depends upon the changes which it undergoes in the soil, the availability of the transformation products to the plant, and the resultant effects of the products on the soil. Therefore, a study of the changes which the material undergoes in the soil is of fundamental importance from a scientific viewpoint.

## REVIEW OF LITERATURE

Pasteur (19) in 1860 was the first to recognize that the transformation of urea to ammonia is brought about by a living organism—*Torula ammoniacale*. It was later discovered that organisms capable of decomposing urea are found in most families of bacteria, actinomyces, and fungi. But certain specific bacteria whose metabolism is closely connected with this substance are called urea bacteria. The optimum temperature for the action of these organisms is about 30°C., and they usually thrive best in a medium made alkaline with ammonium carbonate. However, it has been found that the accumulation of ammonium carbonate from the hydrolysis of the urea is so great in many instances as to kill the organisms themselves.

Microorganisms capable of decomposing urea are found in manure, dust, and water. Miquel (19) found urea organisms in the canal waters of Paris. He also found that the urea organisms of the surface soil were 1 to 2 per cent of the total bacteria, and that manure and urine contain 10 per cent of their flora as urea bacteria. The air of Paris was found to contain one urea splitting organism for every 67 other forms.

Littauer (13) found that the rate of urea decomposition to ammonia was dependent on the soil type, soil moisture, and temperature. He also observed that urea was decomposed more

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<sup>2</sup> Assistant chemist. The writer takes this opportunity to thank Dr. R. W. Ruprecht, and Dr. R. M. Barnette, under whose direction this investigation was conducted, for their helpful suggestions and kindly criticisms, for the facilities made available, and for reading the manuscript. Thanks are also extended to the Synthetic Nitrogen Products Company for providing the funds which made this study possible.

rapidly in a loam soil; and, although drought retarded the rate, increasing the soil moisture above 50 per cent of its maximum water-holding capacity did not cause any significant change in the rate of decomposition.

Brioux (3) states that urea is rapidly transformed into ammonia and acts very much as a physiologically alkaline fertilizer, but later reacts very much as a physiologically acid fertilizer when the ammonia is nitrified.

Lipman and McLean (12) produced experimental evidence to prove that urea manufactured from cyanamid does not give as good results as urea made directly from ammonia and carbon dioxide. They explained this as being due to the presence of dicyanodiamide and guanyl sulfate, which have a toxic effect on plants.

Prince and Winsor (16) investigated the rate of decomposition of urea in cultures containing different percentages of soil and sand. About 10 per cent moisture was used in sand cultures, and 15 per cent in half sand and half soil. The cultures were maintained at room temperature. The index for determining the rate of decomposition of urea was the amount of ammonia present in the cultures at different periods of time. After 5 days they found that only 3 per cent of the urea was converted to ammonia in the sand cultures, 67 per cent in half sand and half soil, and 90 per cent in soil alone.

Bordas and Mathieu (2) investigated the transformation of urea to ammonia by the addition of the enzyme urease (extracted from soybeans) to an aqueous solution of urea. They found that approximately 95 per cent of the urea was hydrolyzed by the urease within 48 hours at 17°C. They also added fermented urine and manure extract to a soil which contained urea. They found increased amounts of ammonia in the presence of these ferments.

The nitrogen transformation in the soil which is most essential for plant growth is the oxidation of ammonia to nitrates. It has been observed that plants absorb nitrogen in the form of ammonia to a limited extent, depending on the stage of growth of the plants. Plant physiologists in general recognize that nitrate nitrogen is the form most available for plant growth.

Smith (18) observed that the optimum soil moisture content for nitrate production from various nitrogenous materials was between 50 and 60 per cent of the maximum water-holding capacity of Norfolk sandy loam soil. However, he obtained a larger nitrate accumulation from urea at 70 per cent of the water-holding capacity of the soil than from ammonium sulfate, dried ground fish, or packing house tankage. He is also of the opinion that after the first period of rapid nitrification the fluctuations of nitrate nitrogen in the soil are due to the action of soil microorganisms rather than to differences in soil moisture, temperature, or reaction.

## OBJECTS OF STUDY

The objects of the present study on urea were to determine the influence of soil moisture, time, concentration of urea in the soil, and temperature, on the rate of transformation of urea to ammonia and subsequently to nitrates. The acidity relationships produced in the soil as the result of accumulated products, ammonia and nitrates, were studied under both unleached and leached conditions of the soil. These studies were carried out altogether in soil cultures.

## EXPERIMENTAL METHODS

### *Method of culture*

All soil cultures were prepared with Norfolk fine sand collected from an uncultivated area which was growing to broomsedge grass. The surface 8 inches of soil was removed, thoroughly mixed, sieved, and dried under atmospheric conditions to air-dry basis.

The cultures were prepared by thoroughly mixing given quantities of urea with 5 kgm. of air-dry soil and placing the mixture in 1-gallon glazed pots, which previously had been weighed. For each pot culture to which urea was applied, a culture was prepared which contained only soil. The calculated amount of water was added to each culture to bring it to the desired soil moisture content. The total weight of the pot, soil, and water being known, the cultures were weighed at periodic intervals and the water lost by evaporation was replaced.

### *Method of sampling*

A representative sample of soil was obtained by making a number of borings from the top to the bottom of each culture with a small metal tube about one-half inch in diameter. Thus several cylindrical portions of soil were removed from different parts of the pot. The sub-samples were thoroughly mixed, and duplicate samples to be analyzed were weighed from the composite. Samples on which the pH was determined were also obtained from this composite.

### *Chemical methods*

Since chemical determinations were made in duplicate on each soil culture, and a large number of cultures were maintained, it was necessary to use relatively simple methods of analysis. Upon investigation there did not seem to be a method for the determination of ammonia in the presence of urea which would meet these requirements. However, three possible methods were selected and compared. The methods were as follows: Distillation with magnesium oxide as described by Emerson (5); Aeration as described by Mathew (14); Displacement of ammonia from the soil by leaching with a salt solution.

Comparable results were obtained with all three of these methods. The aeration method was eliminated, for it became evident that it was not suitable for making a sufficient number of determinations simultaneously.

The results which were obtained by distillation with magnesium oxide did not indicate that urea was broken down by the heat of distillation. However, Emerson (5) suggests that the determination of ammonia in soils by the aid of heat at the boiling temperature is questionable, for some amino acids are usually present and may be decomposed.

It was found to be more convenient to analyze a large number of samples at one time by displacing the ammonia from the soil with a N solution of sodium sulfate. This method consisted of transferring 25-gm. samples of soil to filters and leaching with sufficient N sodium sulfate solution to give 250 cc. of percolate. The percolate was collected in 250-cc. volumetric flasks and thoroughly shaken to insure a uniform distribution of ammonia in solution. Aliquots were removed from the flasks, placed in graduated cylinders, diluted, and nesslerized.

Since this method gave comparable results with sandy soil, and did not involve any process of heat, it seemed to be the more logical method to use.

Nitrates were determined by the phenol disulfonic acid method on aliquots of a 1 to 5 soil extract as described by Schreiner and Failyer (17).

The hydrogen-ion concentration was determined by the quinhydrone electro-metric method in a 1 to 2 soil-water suspension.

#### EXPERIMENTAL RESULTS

##### *Influence of soil moisture and time on the rate of conversion of urea*

The series of cultures used in this experiment were maintained at a constant temperature of 21°C. for 34 days, from February 25 to April 4, 1930. The

TABLE 1  
*Treatment of soil cultures maintained for 34 days at 21°C.*

POT NUMBER	TREATMENT	MOISTURE*
		<i>per cent</i>
1	None	3.80
2	1 gm. urea	
3	None	6.80
4	1 gm. urea	
5	None	9.00
6	1 gm. urea	
7	None	11.70
8	1 gm. urea	
9	None	13.95
10	1 gm. urea	
11	None	15.85
12	1 gm. urea	
13	None	17.95
14	1 gm. urea	
15	None	20.55
16	1 gm. urea	

\* Percentage of moisture on the dry basis of the soil.

treatments of the different cultures, with regard to urea and moisture, are shown in table 1.

Ammonia determinations were made on samples collected from each of these cultures at periodic intervals. The first determinations were made approximately 24 hours after sufficient water was added to adjust the cultures to their respective soil moisture contents. Ammonia was determined as parts per million in a sodium sulfate soil percolate and calculated to milligrams of ammonia per 100 gm. of dry soil. The quantity of ammonia which accumu-

lated from urea was calculated by subtracting the quantity in the check cultures from the quantity in the cultures of the same moisture content and treated with urea.

The results thus obtained on different dates in cultures of the same soil moisture content were averaged by means of a moving average. This was done in order to remove the unevenness between the determinations on different dates, due to causes inherent in the cultures themselves and not dependent on the factors which were under consideration. In the use of the moving average the quantities of nitrogen in each soil moisture content, for three consecutive dates of determination, were added and divided by 3. The average value obtained was considered to represent the quantity of nitrogen as ammonia for the middle date. The average values thus calculated are presented in the ammonia nitrogen plane of figure 1.

One set of surface lines in figure 1 represent the effect of variable soil moisture contents on the accumulation of ammonia at given times. The other set of lines represent the effect of time on the transformation of urea to ammonia in each culture of a given soil moisture content. The rate of accumulation of ammonia from urea was greatest in the soil cultures of low soil moisture contents, and apparently decreased as the soil moisture increased to 13.95 per cent. It again increased up to a soil moisture content of 15.85 per cent and decreased from this point to 20.55 per cent.

The surface lines for given soil moisture contents, time being variable, show that urea was rapidly decomposed to ammonia immediately after application to the soil. And the soil at all moisture contents shows a gradual accumulation of ammonia from urea during the first 20 days of incubation. After 20 days the ammonia content either remained about constant or decreased in all cultures except that of 20.55 per cent moisture.

The soil was approximately saturated at 17.95 per cent moisture and at 20.55 per cent it was submerged under water. Consequently, at 20.55 per cent moisture the biological activity was that of anerobic conditions.

The rate of accumulation of ammonia from urea at the different soil moisture contents is misleading unless the possible oxidation of some of the ammonia to nitrates is considered.

Nitrate determinations were made on soil samples from the cultures previously described at periodic intervals of one week over the period of incubation. The quantity of nitrate which was formed from urea in the different soil moisture contents was calculated as has been described for ammonia and expressed as milligrams of nitrogen per 100 gm. of dry soil.

The results obtained by the moving average are presented in the nitrate nitrogen plane of figure 3.

In figure 3 the heights of the surface lines for given times show that there was no significant increase in the nitrate content of the soil until 17 days after incubation commenced. After 17 days the rate of accumulation of nitrates was comparatively rapid. This is shown by the height of curves for 24 and 31 days

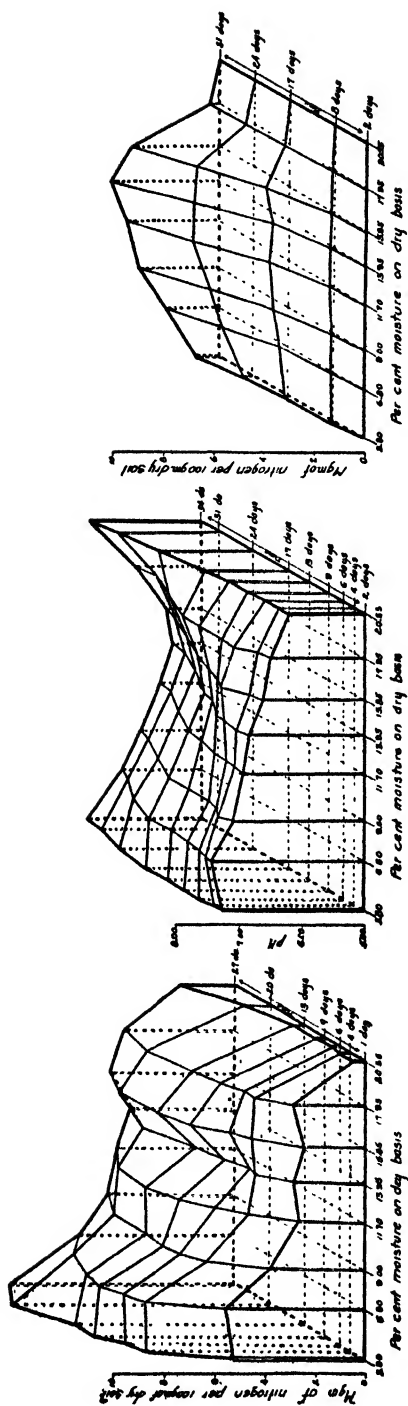


FIG. 1

FIG. 2

FIG. 3

THE EFFECT OF TIME AND MOISTURE ON THE CONVERSION OF UREA TO AMMONIA NITROGEN AND NITRATE NITROGEN, AND THE SUBSEQUENT EFFECT OF THE ACCUMULATION OF THESE PRODUCTS ON THE HYDROGEN-ION CONCENTRATION OF SOIL CULTURES  
(5 kgm. of Norfolk sand and 1 gm. of urea maintained at a constant temperature of 21°C.)

respectively. The rate of nitrification increased directly as the soil moisture increased to 13.95 per cent. From 13.95 to 20.55 per cent moisture the rate of nitrification decreased as the soil moisture increased.

Therefore, 13.95 per cent seems to be the optimum moisture content for the nitrification of urea in Norfolk sand, when the temperature is controlled at 21°C.

In the transformation of urea to ammonia and the subsequent oxidation of the ammonia to nitrate, two counteracting chemical compounds are formed which influence the acidity relationships in the soil. It has been shown that the accumulation of ammonia and nitrate is dependent on the soil moisture content and the time. Consequently, there should be some correlation between the quantities of the respective compounds at the different soil moisture contents and the different times, with the acidity relationships in the soil.

The pH value of the soil was determined on soil samples from each of the cultures previously described on each date that either ammonia or nitrates were determined. The pH values were also averaged by the moving average, and the averaged results are presented in the pH plane of figure 2.

The surface lines for given times show the effect of soil moisture on the pH value of the soil at different times during the incubation period. The surface lines for given soil moisture contents show the effect of time on the pH of the soil for each soil moisture content throughout the period of incubation.

In order to arrive at any conclusions concerning the surface effect of figure 2, both figures 1 and 3 must be correlated with it.

Ammonia, an alkaline compound, and nitrate representing nitric acid; produce converse acidity relationships in the soil. Depending upon the quantities of ammonia and nitrate accumulated in the soil there should be a correlation of the activity of these compounds with the pH value of the soil. The high areas of figure 2 represent the more alkaline condition of the soil, and the lower areas represent the more acid condition. Consequently, there should be a direct correlation between figures 1 and 2, and a corresponding inverse correlation between figures 2 and 3. This correlation holds true in a general way.

Figure 1, which represents the quantity of nitrogen as ammonia in the soil, is high through the area of low soil moisture contents. Figure 3, which represents the quantity of nitrogen as nitrates, is low through the corresponding area; and the pH plane is relatively high through this area. On the other hand, the area of figure 3 which represents relatively high concentrations of nitrogen as nitrate shows a correspondingly lower pH value of the soil through the same area of figure 2. However, at extremely high soil moisture contents, represented by the surface line for 20.55 per cent moisture, the pH value appears relatively high in relation to the quantity of ammonia; but the corresponding line in figure 3 shows that no nitrate was formed under this condition. The indications are that a small amount of ammonia in the absence of nitrates will produce a relatively higher pH value in Norfolk sand of high moisture content than might be expected.



*Effect of increasing the concentration of urea in soil cultures*

The effect of increasing the concentration of urea in the soil was determined by preparing two series of soil cultures. The cultures consisted of equal weights of sieved air-dried Norfolk sand and different quantities of urea. One of the series had the soil moisture contents varied from 10 to 70 per cent of the maximum water-holding capacity of the soil as described by Emerson (5). This series of cultures was placed in the greenhouse where the temperature varied daily. The maximum temperature during the period of incubation, as recorded by a thermograph, was 35°C. while the minimum temperature was about 7°C. The other series prepared in a similar manner, except that the soil moisture was varied from 10 to 60 per cent of its maximum water-holding capacity, was placed in a room with a constant temperature of 21°C. The treatments of the different cultures with regard to urea and moisture are given in table 2.

Nitrates were determined on soil samples from each of these cultures at intervals of 2 weeks over a period of 110 days from October 12, 1929, to January 31, 1930.

The quantity of nitrogen which accumulated as nitrates in the cultures from urea was calculated as previously described.

The results obtained from cultures of 2 gm. and 1 gm. of urea and maintained under variable temperature conditions of the greenhouse are presented in figures 5 and 9, respectively. The results obtained from cultures of similar treatment, but controlled at a constant temperature of 21°C. are presented in figures 7 and 11, respectively.

The pH values were determined on samples from these cultures on the same dates that nitrates were determined, with the exception of the first date after incubation began. The data for these determinations are presented in figures 4, 6, 8, and 10 respectively.

Figures 4, 5, 8 and 9 show the respective pH values and nitrate contents of soil cultures treated with 2 gm. and 1 gm. of urea, respectively, and maintained under greenhouse conditions. Figures 6, 7, 10 and 11 show the respective pH values and nitrate contents of soil cultures treated with 2 gm. and 1 gm. of urea, respectively, and controlled at a constant temperature of 21°C.

There is a correlation between the pH values and the corresponding nitrate contents of the soil. The lines representing the low soil moisture contents range near the bottom of the graphs for nitrogen as nitrate; whereas the corresponding lines of the graphs for pH values range near the top. The inverse correlation between the nitrate content of the soil and its pH value as shown in the three dimensional graphs of figures 2 and 3 is substantiated.

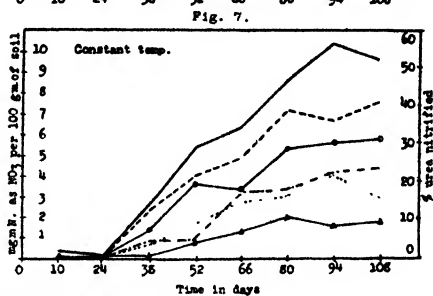
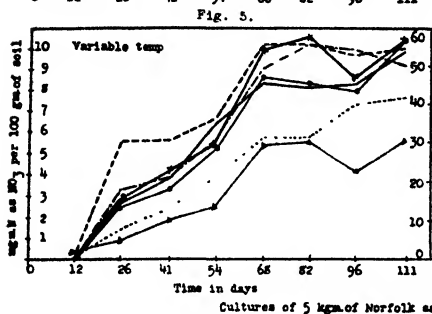
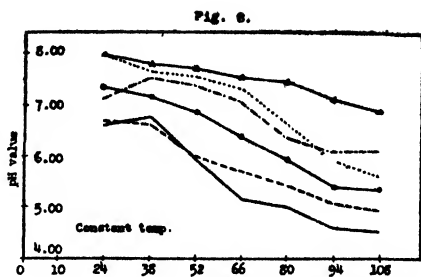
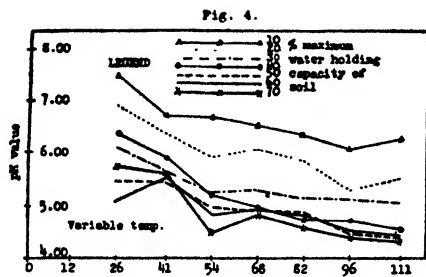
Figures 5 and 9 show the influence of urea concentration on the rate of nitrate accumulation in soil cultures maintained under green-house conditions. Also figures 7 and 11 show the influence of doubling the concentration of urea on the nitrate accumulation in soil cultures controlled at 21°C.

The rate of nitrate accumulation appeared to be slightly more rapid in the cultures treated with twice the amount of urea. However, when the percentage of the urea nitrified is considered, this observation does not seem nearly so significant. The cultures treated with 1 gm. of urea contained 9.2 mgm. of nitrogen as urea per 100 gm. of dry soil. Therefore, in figures 9 and 11, which represent cultures treated with 1 gm. of urea, 9.2 mgm. of nitrogen as nitrate represents 100 per cent nitrification of the urea. Similarly 11 mgm. of nitrogen as nitrate per 100 gm. of dry soil in cultures which received 2 gm. of urea, repre-

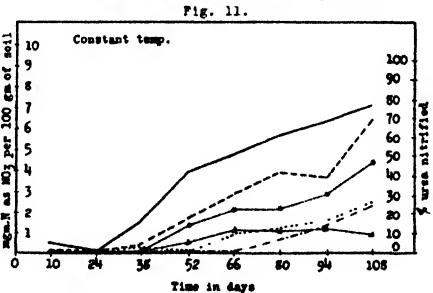
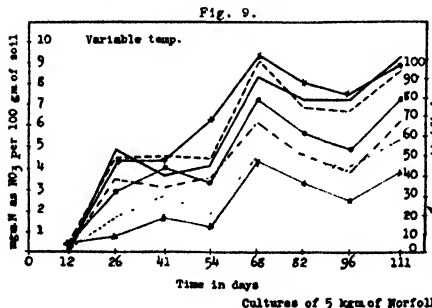
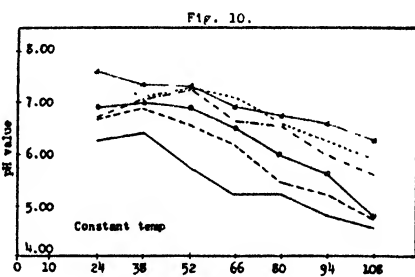
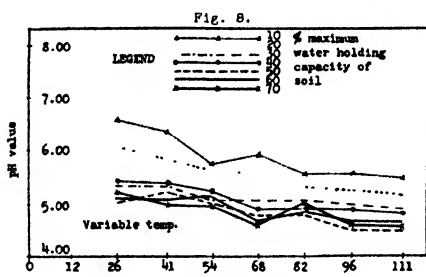
TABLE 2  
*Treatment of soil cultures*

POT NUMBERS		TREATMENT	PER CENT MAXIMUM WATER-HOLDING CAPACITY OF SOIL
Greenhouse conditions	Constant temperature		
1	22	None	10
2	23	1 gm. urea	
3	24	2 gm. urea	
4	25	None	20
5	26	1 gm. urea	
6	27	2 gm. urea	
7	28	None	30
8	29	1 gm. urea	
9	30	2 gm. urea	
10	31	None	40
11	32	1 gm. urea	
12	33	2 gm. urea	
13	34	None	50
14	35	1 gm. urea	
15	36	2 gm. urea	
16	37	None	60
17	38	1 gm. urea	
18	39	2 gm. urea	
19		None	70
20		1 gm. urea	
21		2 gm. urea	

sents 60 per cent of the 18.4 mgm. of nitrogen added as urea per 100 gm. of dry soil. Consequently the graphs which represent an application of 2 gm. of urea show that only a little more than half as much of the urea was converted to nitrate as in the cultures of half the concentration, when considered on the basis of the percentage of the urea nitrified.



### THE EFFECT OF TEMPERATURE AND MOISTURE ON THE CONVERSION OF UREA TO NITRATES AND THE RESULTANT EFFECT ON THE pH OF SOIL CULTURES



### THE EFFECT OF TEMPERATURE AND MOISTURE ON THE CONVERSION OF UREA TO NITRATES AND THE RESULTANT EFFECT ON THE pH OF SOIL CULTURES

*Influence of temperature on rate of nitrate nitrogen production in soil*

A number of soil investigators have observed that temperature is a factor which controls, in a great measure, the quantity of nitrates produced in unit time. Greaves (7) points out some observations made by Schlösing, as follows: "Schlösing found nitrification very slow at 7.5°C.; quite marked at 11°; reached its maximum at 37°, and ceased entirely at 55°."

Panganiban (15) found that nitrification processes take place between 15° and 40°C. and that the optimum temperature in soil cultures is about 35°C. or slightly higher.

The effect of two temperature conditions on the accumulation of nitrates from urea, is represented by figures 5 and 7 for 2 gm. of urea; and by figures 9 and 11 for 1 gm. of urea. The temperatures used were a constant temperature of 21°C. and the variable temperature of the greenhouse. The 1 gm. urea cultures were selected to show the relation between temperature and nitrate nitrogen accumulation in the soil. The nitrate nitrogen determinations of different soil moisture contents were averaged for each date of determination. This gave a mean nitrate nitrogen accumulation for all the cultures of different soil moisture contents represented in figures 9 and 11. The results calculated for the constant temperature of 21°C. and the variable temperature of the greenhouse are plotted in figure 12.

The thermograph readings showed that the temperature in the greenhouse varied from a maximum of 35°C. to a minimum of 7°C. This was a temperature range of from 14°C. higher to 14°C. lower than the constant temperature of 21°C. The maximum and minimum temperature range alone has little significance in comparing the nitrate accumulations under the two temperature conditions. The length of time that the temperature of the greenhouse ranged higher or lower than 21°C. would be expected to produce a corresponding effect on the nitrate accumulations under that condition.

The average daily temperature of the greenhouse was obtained by tabulating the temperature as recorded on thermograph charts at each 2-hour period of the day. These were added and divided by 12. The quotient was taken as the average temperature of the greenhouse for each day. The data for the averaged daily temperature as calculated are also plotted in figure 12.

Figure 12 shows that the nitrate accumulation in cultures of the greenhouse were much higher than those of the constant temperature condition, but the daily temperature of the greenhouse was not on the average significantly higher than 21°C. However, the 14-day interval between the first and second nitrate determinations show a relatively higher average daily temperature and a correspondingly more rapid nitrate accumulation for greenhouse conditions. Subsequent to the second determination the average daily temperature of the greenhouse was distinctly lower than 21°C., and the nitrate accumulation during this period was practically at a standstill. Between the third and fourth determinations the average daily temperature of the greenhouse was higher than

21°C., and the nitrate accumulations increased rapidly. The next two periods of observation show a distinctly lower average daily temperature in the greenhouse. The result was that the nitrate nitrogen actually decreased in the soil cultures. The final period of observation shows a gradual increase in the average daily temperature, and there was a corresponding increase of nitrate nitrogen in the soil cultures.

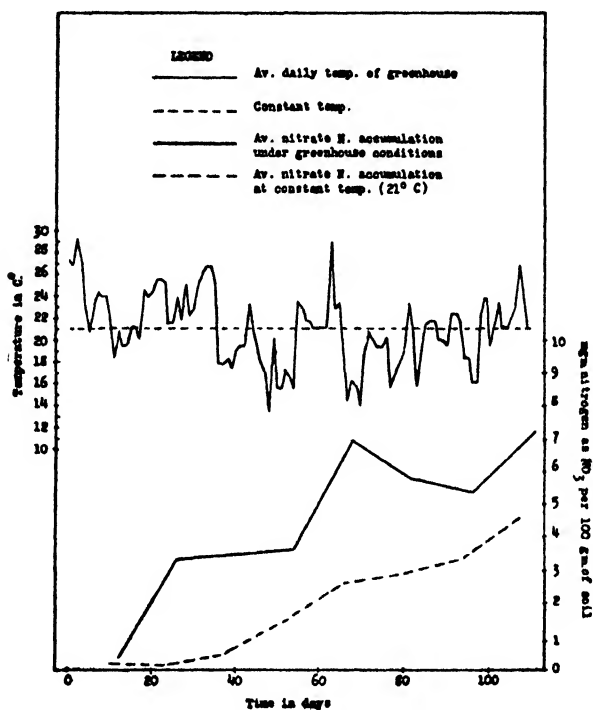


FIG. 12. COMPARISON OF VARIABLE TEMPERATURE OF GREENHOUSE AND CONSTANT TEMPERATURE OF 21°C. ON THE RATE OF CONVERSION OF UREA NITROGEN TO NITRATE NITROGEN

The nitrogen graphs are an average of all nitrate nitrogen accumulations of different moisture contents as shown in figures 9 and 11 respectively. (5 kgm. Norfolk sand and 1.00 gm. urea.)

The indications are that the higher temperature of the greenhouse, immediately after incubation began, stimulated a rapid conversion of the urea to the nitrate form. Consequently, even though lower temperatures than 21°C. tended at times even to decrease the amounts of nitrate nitrogen in these cultures, the gains made in the early periods of incubation were never overcome by the cultures maintained at 21°C. The cultures maintained at 21°C. show a progressive increase of nitrates throughout the period of incubation.

*Effect of leaching the soluble salts from the soil*

Crowther (4) and others have shown that the total soluble salts in a soil affect its pH value depending on the concentration and chemical nature of the salts.

It was observed by Brioux (3) and has been illustrated in figures 1, 2 and 3 that the accumulation of ammonia from urea increased the pH value considerably. On the other hand the accumulation of nitrates decreased the pH value of the soil. However, soil cultures are not comparable to field conditions where

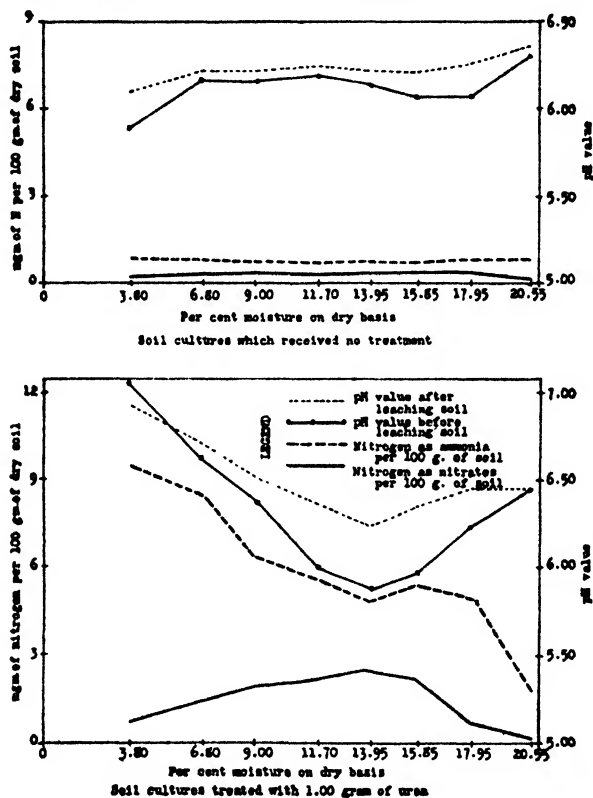


FIG. 13. EFFECT OF LEACHING THE SOLUBLE SALTS FROM THE SOIL ON ITS pH VALUE AS CORRELATED WITH THE AMMONIA AND NITRATE NITROGEN CONTENT OF THE SOIL

the accumulated products are removed either by the absorption of growing plants or by leaching due to rains. Therefore, it was thought desirable to leach soil samples free of their soluble salts and determine the residual effects of the added urea on the soil. The pH value of the soil was used as an index of the residual effects.

Samples for this observation were obtained from the soil cultures described in table 1. At periodic intervals of about 1 week 25-gm. samples of soil from each

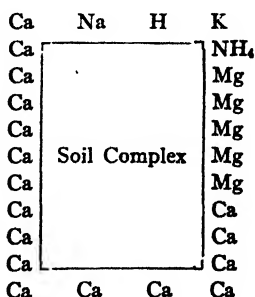
of the cultures were placed on filters and leached with distilled water so as to give 250 cc. of percolate. This quantity of water was found to be sufficient to remove practically all the nitrate salts from the soil. A portion of the soil, after leaching, was removed from the filters, and its pH value was determined by the quinhydrone electrometric method. The pH value was also determined on samples removed from the cultures at the same time but not leached with water.

The pH values of the soil from cultures of each soil moisture content, determined on different dates, were averaged.

Ammonia and nitrate determinations were also made on the cultures during the period of incubation. The intervals of determining the ammonia, nitrates, and pH before leaching are indicated on the time axis of the three dimensional graphs of figures 1, 2, and 3. The averaged results are plotted in figure 13. The curves in figure 13 for the cultures treated with 1 gm. of urea, with the exception of the curve for the pH after the soil had been leached, represent a mean of the surface lines in figures 1, 2, and 3 for given times.

The correlation represented in figure 13 is most easily explained by assuming certain physico-chemical relationships between the colloidal material and soluble salts of the soil. The precise mechanism of this relationship has been the subject of much controversy largely devoted to attempts to demonstrate the chemical or physical nature of the reactions. However, the phenomenon is probably a case of base exchange. Hissink (8), Gedroix (6), Wiegner (20), Kelly and Brown (10), and others have shown that the exchange of cations between soils and salt solutions takes place with great rapidity. The exchangeable ions are regarded as resulting from the ionization of the surface molecules of the colloidal or soil complexes. The surface of the soil complex is considered as forming a double layer probably comparable to the Helmholtz double layer with the cations loosely held in the outer layer of the liquid phase. It has also been observed that certain cations are more strongly held than others, depending on their chemical nature.

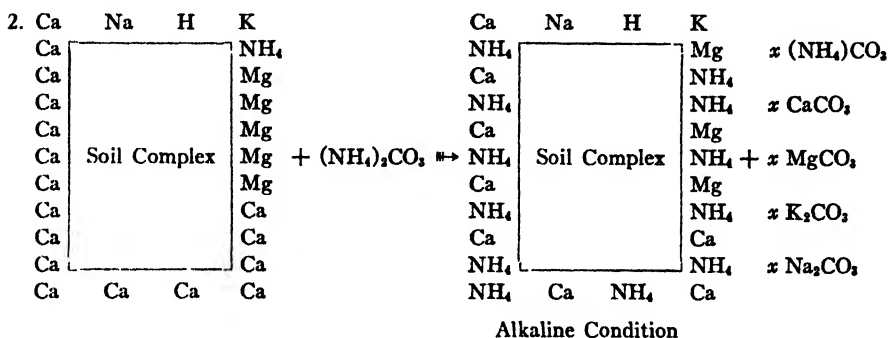
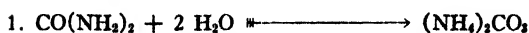
The absorbing complex may be assumed to be represented by the following symbol:



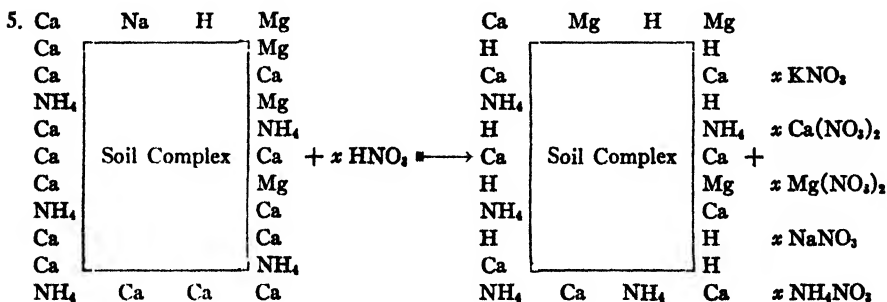
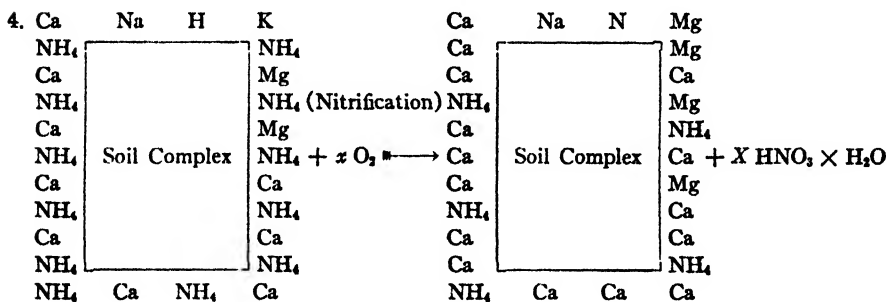
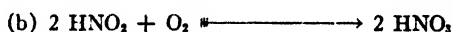
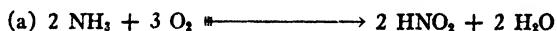
The foregoing symbol represents approximately the proportion of replaceable Ca, Mg, Na, and K as determined by Barnette and Hester (1) in a similar soil to that used in this experiment, with regard to soil type and pH value.

However, the proportion of the different cations absorbed by the soil complex has been observed to be dependent on the chemical composition of the soil colloids, the acidity relationship of the soil, and the chemical properties of the cations.

The reactions which urea undergo as the result of the biological activity and the physico-chemical equilibria of the resulting products with the soil are represented as follows:



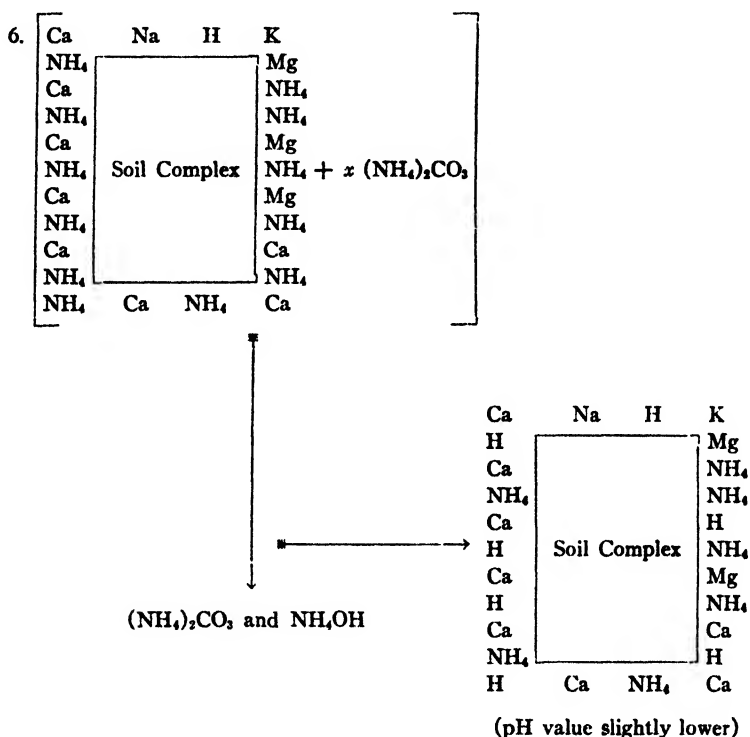
### 3. Process of nitrification





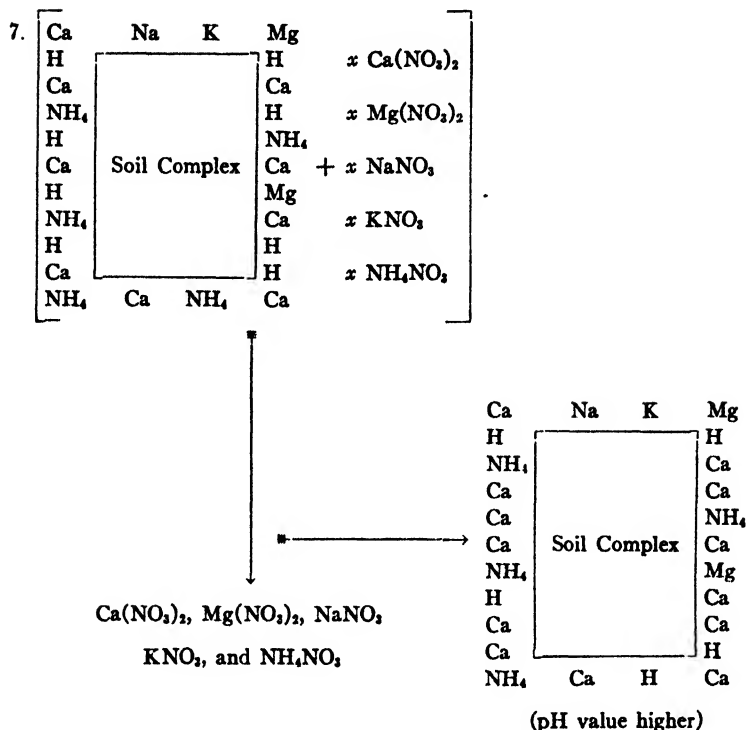
Equations 2, 4, and 5 are assumed to represent in a general way the physico-chemical equilibria between the soil colloids and the chemical compounds present in the soil at different stages of biological activity in the decomposition of urea.

Equation 6 represents a possible explanation for the lowering of the pH value of the soil of low moisture content, where the ammonia content is high and the soluble nitrate content is low. Equation 2 represents the soil complex equilibrium before leaching, and 6 after leaching with water.



The indications are that the removal of some of the ammonia, probably as ammonium carbonate and ammonium hydroxide, from the soil of high ammonia concentration and low nitrate content decreased the pH value of the resultant soil complex in water.

The portion of figure 13 where the pH value of the soil is increased considerably as the result of leaching, is represented by equation 7. Equation 5 represents a similar condition of the soil before leaching.



It is well known among soil workers that the addition of a neutral salt to a suspension of an acid soil liberates a considerable amount of acid, which can be estimated by titration of the filtrate as in the "lime requirement" method of Hopkins (10).

Crowther (4) has shown that the treatment of soils with salt solutions in the laboratory and subsequent leaching with water causes a considerable increase in the pH value of the soil. This observation is borne out in figure 13 for neutral salts which were not added to the soil but were present as the result of biological changes of urea.

The accumulation of relatively large quantities of soluble salts in the soil, resulting from the oxidation of ammonia to nitrate at favorable moisture contents (ranging from 6.80 to 17.95 per cent moisture), decreased the pH value of the soil. Subsequently, the leaching of these soils and the removal of most of the soluble salts gave an equilibrium of the soil with water which was significantly less acid than that of the soil-water-salt equilibria. At 20.55 per cent moisture the nitrate content indicates small quantities of soluble salts, and consequently the pH value was not changed appreciably as the result of leaching.

Further evidence which bears out this relationship between the quantities of different forms of nitrogen in the soil and the extent to which the pH value of the soil is increased as the result of leaching, is shown by the untreated soil at

the top of figure 13. The quantities of nitrogen as ammonia and nitrate in the soil are fairly constant. Consequently the pH values of the soil before and after leaching show a corresponding difference which is fairly constant.

#### SUMMARY

Ammonification and nitrification studies of urea were made by mixing given quantities of urea with 5 kgm. of air-dried Norfolk sand. Observations were made on the influence of soil moisture, time, concentration of urea in the soil, and temperature, on the conversion of urea nitrogen to ammonia and nitrate nitrogen. The acidity relationships produced in the soil as the result of accumulated products from urea, ammonia, and nitrates, were studied under both leached and unleached conditions of the soil.

A correlation was found to exist between the soil moisture content and the rate of transformation of urea to ammonia and subsequently to nitrates in Norfolk sand. Apparently the rate of ammonia accumulation from urea decreased with an increase in the soil moisture in the early period of incubation. Later when nitrification began the quantity of ammonia, accumulated from urea, decreased with an increase in soil moisture up to 13.95 per cent on the dry basis of the soil. At 15.85 and 17.95 per cent moisture the ammonia accumulation again increased. At 20.55 per cent moisture, which represents an anerobic condition of the soil (saturated with water), very little ammonia accumulated from urea.

The ammonia formed from urea was converted to nitrate nitrogen very slowly in comparison with the rate of conversion of urea to ammonia. There was no significant production of nitrate nitrogen at 21°C. until 17 days after urea was added to the soil. The optimum soil moisture for nitrate production in Norfolk sand controlled at 21°C. was found to be 13.95 per cent. No nitrates were formed in the culture of 20.55 per cent moisture, which represents anerobic conditions.

The acidity relationships of the soil were counteracted by the two nitrogen transformation products from urea. Ammonia nitrogen increased the pH value, and nitrate nitrogen decreased the pH value of Norfolk sand in soil cultures.

Doubling the concentration of urea in the soil produced a slightly higher concentration of nitrate nitrogen, but calculations for the percentage of urea nitrogen converted to nitrogen as nitrate showed that the conversion was less efficient at the higher concentration of urea.

Over an average daily temperature range varying between 10°C. and 30°C., the fluctuations in the production of nitrates from urea seemed to vary directly with the temperature. At a constant temperature of 21°C. the rate of production was slow at first but increased gradually throughout the experiment.

Leaching the soluble salts, which were produced in the soil from urea as the result of biological changes, increased the pH value of the soil-water suspension. The degree of the change in pH value as the result of leaching was found to be dependent on the concentration of ammonia and nitrate nitrogen in the soil.

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# BORON REQUIREMENTS OF COTTON

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Among those elements which, in addition to the older list of 10 essential elements, are now considered essential to the growth of many if not all plants, boron has received a fairly generous share of attention. In demonstrating the essential nature of this widely distributed element, emphasis has been placed upon the use of specially purified chemicals and upon the use of culture vessels free of boron. The requirements of some plants have been shown to be satisfied when boron is available in the nutrient solution in concentrations considerably below 1 p.p.m. The boron requirements of cereals, if boron is actually essential to their growth, is much lower. The requirements of certain other plants, however, are not satisfied by what may be called traces of boron and, as is shown in table 1, maximum growth and fruitfulness of cotton occur only when this element is supplied in concentrations of 10 p.p.m. in the nutrient solution. An optimum concentration of this magnitude and the concentrations found in the resulting plant material suggest that boron plays a major rôle in cotton metabolism. It has been found that the boron requirement of cotton is among the highest of some 60 plants which have been compared.

In addition to its essential character, boron, when present in excessive concentrations, is highly toxic. This fact has been known for a long time, but only more recently has it been shown that its excessive occurrence (0.5 p.p.m. and upward<sup>2</sup>) in the irrigation waters in a number of areas in arid regions is the cause of certain abnormal symptoms and depressed growth which previously were attributed to other factors. For the past several years the occurrence and effects of boron in irrigation water have been the object of investigations by the U. S. Department of Agriculture, through the Division of Western Irrigation Agriculture. These investigations have disclosed marked differences in the tolerance of different plants to boron and, incidental to the investigations, a wide variability in the requirements of different plants. A number of crop plants have responded favorably to boron concentrations sufficiently high to produce injury or inhibit the growth of boron-sensitive plants. The spread between the suboptimum and excessive concentrations

<sup>1</sup> Rubidoux Laboratory, Riverside, California.

<sup>2</sup> The boron concentrations in soil solutions normally exceed those of the irrigation water after prolonged use. Only the more sensitive plants are injured by boron concentrations in irrigation waters as low as 0.5 p.p.m.

for some plants has been found to be so narrow that an actual overlapping of beneficial and injurious effects is indicated. Boron accumulates in leaves with age, and strikingly different concentrations exist in the different plant organs and in different portions of the same organs.

The data on boron relations of cotton as here presented are selected from information being obtained with respect to a number of crop plants in an investigation of the boron problem under western irrigation conditions. The completion of these investigations is not immediately in sight and for that reason it has seemed desirable to call attention to the response of cotton to boron in order that the information might be available in other investigational fields. Much of the earlier work on boron in relation to plant growth has been

TABLE 1  
*Effect of boron on growth and yield of acala cotton plants*

CONCENTRATION OF BORON IN CULTURE SOLUTIONS— <i>p.p.m.</i>	"0"	1	5	10	15	25
<i>1929</i>						
Mean weight of 4 plants. . . . . <i>gm.</i>	114	. .	149	177	154	83
Green bolls on 4 plants, December 9 . . . .	27	. . . .	8	3	6	8
Open bolls on 4 plants, December 9. . . . .	1	. . . .	22	30	25	11
Weight seed cotton in open bolls. . . <i>gm.</i>	5	. . . . .	170	241	201	88
Boron—entire plants,* . . . . . <i>p.p.m.</i>	12	. . .	123	312	361	536
Boron per plant,* . . . . . <i>mgm.</i>	1.4	. . .	13 1	36 4	37.3	32 8
<i>1930</i>						
Mean weight of 4 plants . . . . . <i>gm.</i>	51	124	150	195	110	133
Green bolls on 4 plants, November 14 . . . .	3	17	25	34	21	17
Open bolls on 4 plants, November 14 . . . .	0	5	3	3	2	4
Boron—leaves only . . . . . <i>p.p.m.</i>	16	187	306	522	833	1,625
Boron—stems and roots only. . . . . <i>p.p.m.</i>	15	24	32	33	48	60
Boron per plant† . . . . . <i>mgm.</i>	0 7	6 4	12 4	26.4	25 4	53.6

\* Seed cotton not included.

† Burs and seed cotton not included.

of a qualitative, or exploratory, character, in that only a few investigators have undertaken accurate boron determinations to observe either the rate of absorption of boron from nutrient solutions or the extent to which the toxic or beneficial effects of boron are associated with its accumulation and distribution in the plant.<sup>3</sup>

The experimental plants discussed were grown in large sand beds<sup>4</sup> so equipped with plumbing, supply barrels, and drains that the nutrient solution held by the sand could be reestablished each day by flooding and draining to maintain the concentration of boron and at the same time provide like conditions for the different plants undergoing comparison in each bed.

<sup>3</sup> Acknowledgment is made to Mr. L. V. Wilcox for the boron determinations herein reported.

<sup>4</sup> F. M. EATON. 1931 A large sand culture apparatus. *Soil Sci.* 33: 235-240.

In 1929 five boron concentrations were employed; namely, "0", 5, 10, 15, and 25 p.p.m., and in 1930 a 1 p.p.m. concentration in addition. The concentrations are in terms of elemental boron supplied as boric acid. The nutrient solutions contained (in addition to boron, iron, and manganese) calcium nitrate, magnesium sulfate, and potassium acid phosphate in concentrations of 6, 3, and 3 millimoles per liter respectively. As a result of impurities of the "C. P." chemicals employed and of the free exposure of the beds out of doors, the "0" bed solution contained approximately 0.05 p.p.m. of boron.

Each of the more important crops compared has been grown during at least two seasons to compensate in part for the necessarily limited number of plants involved.

Table 1 compares the growth and fruitfulness of Acala upland cotton grown in the different beds during each of two seasons. In both years the plants in the "0" bed shed most of their floral buds and bolls. Only one undersized open boll was produced in this bed in 1929, and in 1930 no bolls set previous to October were retained. The plants of the "0" bed were shorter than those of the 1 or 5 p.p.m. beds and the branch lengths were reduced. The leaves of the "0" bed cotton were characterized by mildly arrested development of the veins resulting in some buckling of the mesophyll, by marked irregularities in shape, and by large irregular areas which died out after having become chlorotic.

No deficiency symptoms could be noted in the plants supplied with 1 p.p.m. of boron and except for their smaller size and lighter crop, as compared with higher concentrations, they would have been considered normal.

The largest plants and the greatest number of bolls resulted in each season in the culture bed supplied with nutrient solution containing 10 p.p.m. of boron. The plants of the 10 p.p.m. bed, producing as they did the highest yield, showed very mild but definite burning of the leaves, both marginal and in intravenous spots adjacent to the margins. This type of burning, commonly preceded by yellowing, which is characteristic of boron injury, did not occur in the 5 p.p.m. concentration. The concentration of boron, other than in the "0" bed, did not influence the weight of individual bolls or the seed cotton per boll.

As shown in table 1, 1930, the boron concentrations in the leaves greatly exceeded that of the stems and roots, and these concentrations increase as the boron supplied is increased.

The burs were not included in the analyses of the 1930 plants but in 1929, when boron determinations were made on entire plants, exclusive of seed cotton, the cotton of the 10 p.p.m. bed accumulated 36.4 mgm. of boron per plant. An acre of 14,000 such plants under similar conditions would have removed from the soil 510 gm. of boron, or 5.2 pounds of anhydrous sodium borate, or 9.9 pounds of 10 hydrate borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ).

When borax is applied to a soil only a portion of it remains dissolved in



the soil solution. If the soil to which it is added is low in boron a very considerable part of it may be rendered unfree, or insoluble, by the soil. In contrast with nitrates, which are readily removed from soil by one or two leachings, a number of successive percolates or extractions are required to recover a major part of boron similarly added. The extent to which boron is rendered partially insoluble by different soils varies greatly. For these reasons it cannot be stated in advance how much boron would have to be added to a particular soil to produce a soil solution of any desired concentration.

During and following the War considerable injury resulted when potash salts containing boron were used as fertilizers in the cotton belt and elsewhere. This injury was the cause of serious concern on the part of farmers and was made the subject of investigations, both by a number of the state experiment stations and by the U. S. Department of Agriculture.<sup>5</sup> These reports show that the higher concentrations used caused serious injury, but for cotton the effects of the smaller applications were uncertain. Heavy rains following the applications served to lessen the injury. Two features of these investigations are of primary interest here: (a) The borax in those tests was either placed directly in the drill rows or was broadcast above the drill rows at or near the time of planting, and (b) adequate studies are not reported of the residual effects of the boron on yields during succeeding years. An element as toxic as boron would be expected to produce highly injurious effects when concentrated in a restricted portion of the root zone and it is surprising that applications of 10 pounds of anhydrous borax to the acre made in this way did not produce injurious effects in all of the tests.

Indirect evidence tending to support the view that applications of boron under field conditions may have a beneficial effect upon cotton is found in the results of nitrate fertilizer trials at the Delta Experiment Station of Mississippi. The Delta Station Service Sheets No's. 23 and 29 show that the continued use of Chilean sodium nitrate has resulted in higher yields than those resulting from synthetic nitrate fertilizers. Five-year averages show an increased yield of the sodium nitrate plats over calcium nitrate plats of 172 pounds of seed cotton to the acre and nine-year averages show sodium nitrate to have increased the yield by 32 pounds over ammonium nitrate. Of the latter it is stated in footnote that for the years 1926-27-28-29 the average increase for sodium nitrate was 788 pounds an acre, for ammonium sulfate 498 pounds, and for cyanamid 528 pounds. Analyses made by this laboratory have shown two samples of Chilean sodium nitrate to have contained respectively 298 and 176 p.p.m. of boron and the producers report an analysis showing 791 p.p.m. of boron. It is probably natural that variability should be shown in the impurities in a product of this character. A sample of a newer, more refined, form of Chilean sodium nitrate, the Champion Brand, contained by our analysis 76 p.p.m. of boron and the latter of the Service Sheets cited indicates a

<sup>5</sup> SKINNER, J. J., BROWN, B. E., AND REID, F. R. 1923 Effect of borax on the growth and yield of crops. U. S. Dept. Agr. Dept. Bul. 1126.

slightly lower yield when it is used as a fertilizer. Each of the few determinations on synthetic nitrate fertilizers made by us have shown these products to be essentially free of boron. Although relatively small additions of boron to boron deficient soils might produce outstanding effects, this explanation of an apparent advantage resulting from the boron carried by nitrate of soda is nevertheless inconclusive, since annual applications of 200 pounds of material carrying 790 p.p.m. of boron would add to the soil but 72 gm. of boron a year.

The experimental evidence here presented is believed to indicate that gratifying results might follow the use of boron as a fertilizer in cotton field tests. In those localities where increased yields might result, the optimum concentrations would doubtless be relative to the physical characteristics of different soils and their initial boron concentrations. It is believed that boron used as a fertilizer should be incorporated in the soil well in advance of planting and it is possible that boron in a form more slowly soluble than borax, such as colemanite ( $2\text{CaO} \cdot \text{B}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) might be most desirable. Some of the winter crops, such as Melilotus, Winsor beans, and vetch, are highly tolerant to boron, as are alfalfa and beets. Boron should not be applied immediately before corn, other cereals, or beans, since a growth depression of 25 per cent or more can be expected at soil solution concentrations optimum for cotton.



# SOLUBLE ALUMINUM STUDIES: II. MINIMUM CONCENTRATIONS OF ALUMINUM FOUND TO BE TOXIC TO CORN, SORGHUM, AND BARLEY IN CULTURE SOLUTIONS<sup>1, 2</sup>

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It has been shown in some previous work reported from this laboratory (17), as well as in some earlier work by Magistad (11) and by Pierre (16), that the concentrations of soluble aluminum in the displaced solution of acid field soils are comparatively low. Therefore, in determining the validity of the toxic aluminum theory of soil acidity, a question of prime importance is: are these relatively low concentrations of soluble aluminum found in acid soils sufficiently high to be injurious to the growth of plants?

The general problem of determining the concentrations of aluminum required to be toxic to plant growth has been studied quite extensively by means of culture solutions (2, 3, 8, 9, 10, 18, 20). The results obtained, however, are very conflicting and in most cases their interpretation is difficult. This is mainly because of the failure of many investigators to recognize the various factors which affect the solubility of aluminum and also the secondary effects of additions of aluminum salts to culture solutions. Line (7) and Gile (5) have discussed some of these factors in considerable detail and have questioned many of the experiments upon which the toxic aluminum theory of soil acidity is based.

Among the main criticisms of the culture-solution investigations on aluminum toxicity may be listed the following:

Failure to maintain constant the H-ion concentration of the culture solutions.

Failure to maintain the desired aluminum concentrations or to make determinations of the concentration of aluminum found in solution.

Failure to consider the mutual precipitating action of aluminum and phosphate in the culture solution.

Growing the plants in very small culture vessels and harvesting the plants at too early a stage of growth.

It is noteworthy that in none of the experiments which have been reported were determinations made of the concentration of aluminum actually present in the culture solution during the growth of the plants. This no doubt is due partly to the fact that until recently there was no accurate method for determining small amounts of aluminum. The solubility of aluminum is primarily a function of the hydrogen-ion concentration. It is well known, however, that plants may rapidly alter the pH of the culture solutions. This is especially true if the culture vessels are small. Since a rise in pH readily causes precipitation of aluminum, it is evident that the pH values of the cultures should be accurately controlled. Moreover, since it has been shown that aluminum phosphate is precipitated even at low pH values, the addition of aluminum salts to complete nutrient solutions may cause the precipitation of aluminum as well as of phosphate. Thus it is possible that in such cases the poor plant growth which results may be due to phosphate starvation rather than to aluminum toxicity.

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<sup>1</sup> Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 112.

<sup>2</sup> Contribution from the Department of Agronomy and Genetics.

McLean and Gilbert (9, 10) in their experiments recognized the precipitating action of aluminum on phosphate and prevented this from taking place by alternating their plants between complete nutrient solutions containing phosphate and complete nutrient solutions minus phosphate but containing aluminum. For this reason their results are probably the most satisfactory that have been reported regarding the minimum concentrations of aluminum found toxic to the growth of plants. Their minimum toxic concentrations, however, were in many cases considerably higher than the concentrations ordinarily found in the displaced solution of acid field soils, as will be discussed more fully later. Magistad (11) in a comprehensive investigation grew a number of different plants in sand cultures to which was added Hartwell and Pember's nutrient solution, with and without aluminum, adjusted to different pH values. Since the culture vessels were small, it was difficult to maintain the reactions of the culture solutions closer than to within 0.4 to 1.0 pH unit even though they were adjusted twice daily. Moreover, iron phosphate was mixed with the sand to furnish additional phosphorus; and, since no determinations were made of the amount of aluminum remaining in solution, it is not possible to be sure of the concentrations of aluminum present in the cultures. The results obtained, however, indicate that relatively low concentrations of aluminum may be toxic to plant growth.

In many of the other investigations where aluminum was found toxic to plants in culture solutions (1, 3, 4, 6, 12, 18) the experiments were planned primarily to prove the toxicity of aluminum rather than to determine very accurately the minimum concentrations required to cause injury. These and other investigations previously mentioned will not be discussed here because they are difficult to interpret accurately and because Line, Magistad, and others have already made critical reviews.

The objectives of the present investigation were: first, to study the relative effect of hydrogen-ion concentration and aluminum concentration on plant growth; and second, to determine the minimum concentrations of aluminum found injurious to three crop plants which have been reported as showing different degrees of tolerance to soluble aluminum.

#### METHODS OF PROCEDURE

The three kinds of plants used in this study were barley, sorghum, and corn. These plants are classified by McLean and Gilbert (9) as being sensitive, medium sensitive, and resistant to soluble aluminum, respectively. Since Hartwell and Pember's nutrient solution (6) has been extensively used in aluminum studies by other investigators and since it has the advantage of resembling the soil solution in phosphate concentration more than do most nutrient solutions, it was used in this investigation with slight modifications. The modifications consisted in omitting the ferric nitrate, in adding 0.25 p.p.m. of boric acid and 0.38 p.p.m. of manganese sulfate, and in substituting monopotassium phosphate for mono-calcium phosphate in equivalent amounts. In order to prevent precipitation of the aluminum as aluminum phosphate, a method was used similar to that of McLean and Gilbert (9, 10) of alternating the plants between complete nutrient solutions and similar solutions containing no phosphorus but containing aluminum. The plants were changed from one of these series to the other each day instead of twice a week, as was done by these investigators. Once a week, or oftener, if the plants appeared to need iron, they were put in a third series of pots containing Hartwell and

Pember's nutrient solution minus the phosphate but containing 2.77 p.p.m. of iron as the tartrate and kept at pH 4.5. Distilled water was used except for the iron series of cultures, where tap water was employed. New culture solutions were made up about every three weeks during the progress of the experiment.

Six different treatments were studied, and the cultures were in triplicate. In order to keep the aluminum in solution, the pH values of all the cultures, except those of treatment 1, were kept at 4.5. The cultures of treatment 1 were kept at pH 6.0 as a control on the effect of hydrogen-ion concentration on the growth of the plants. Treatments 2 to 6 inclusive, received 0, 1, 2, 5, and 10 p.p.m. of aluminum, respectively. Determinations of the pH value of the solutions were made daily, and the solutions were adjusted, if necessary, by additions of sodium hydroxide. Phosphate and aluminum determinations were made at frequent intervals, and additional amounts of potassium phosphate were added when found necessary to maintain the original concentration of phosphate. Aluminum was determined by a slight modification of the "aluminon" method (17), and phosphorus was determined by the blue colorimetric method (14).

The culture vessels were glazed earthenware pots of approximately 15-liter capacity. They were covered with galvanized lids containing three holes of such size as to hold paraffined corks 2.5 inches in diameter. Each cork contained three holes in which the plants were held upright by means of non-absorbent cotton.

The corn, barley, and sorghum were germinated in clean quartz sand, and the corn seedlings were transferred to the culture solutions on August 30; the barley seedlings, on September 3; and the sorghum seedlings, on September 8. The three crops were grown together during the first part of the experiment, each culture vessel receiving three seedlings of each plant. Later the number of seedlings in each cork was reduced to two. When the corn plants grew large enough to shade the barley and sorghum, they were removed to separate culture vessels, and each plant was placed in a separate cork. Later, when the barley and sorghum plants appeared too crowded, they were also placed in separate culture vessels.

Notes of the plants were taken as differences became apparent, and just prior to harvesting, photographs of the crops were taken. The corn plants were harvested on October 10, and the barley and sorghum plants were cut on December 6.

## RESULTS OF INVESTIGATION

### *Control of nutrient solutions*

The procedure which has just been described made possible an accurate control of the phosphorus, aluminum, and hydrogen-ion concentration of the culture solutions. This is shown for aluminum and hydrogen-ion concentra-

tion in table 1. The pH values of the solutions kept at pH 4.5 were found to decrease only very slightly during a 24-hour period, the maximum daily change being in most cases less than one-tenth of a pH unit. The various concentrations of aluminum in the culture solutions were also well maintained except where 10 p.p.m. of aluminum had been added. It will be noted that during the period between September 8 and October 3, the average amounts of aluminum found in solution in cultures 6A, 6B, and 6C were less than in cultures 5A, 5B, and 5C, where only half as much aluminum had been added. This was found to be due to some silica in the sodium hydroxide used to adjust the reaction of the culture solutions. Aluminum silicate is precipitated even at relatively low pH values and since more alkali was necessary to correct the acidity formed when the largest amount of aluminum was added, less alumi-

TABLE 1

*Treatment of cultures and analyses of solutions with respect to aluminum and hydrogen-ion concentration during various periods of plant growth*

CULTURE NO	TREATMENTS		ANALYSES OF CULTURE SOLUTIONS*			
	Aluminum	H-ion concentration	Sept. 8-Oct. 3		Oct 16-Nov 14	
			H-ion concentration	Aluminum	H-ion concentration	Aluminum
	p p m.	pH	pH	p p m.	pH	p p m.
1. A, B, C	None	6 0	5 69	0	5 79	0
2. A, B, C	None	4 5	4 38	0 03	4 47	0 02
3. A, B, C	1 0	4 5	4 41	1 03	4 46	1 07
4. A, B, C	2 0	4 5	4 36	2 28	4 41	2 47
5. A, B, C	5 0	4 5	4 39	4 56	4 44	4 64
6. A, B, C	10 0	4 5	4 40	4 06	4 42	7 55

\* The values given are averages of daily determinations in the case of pH values; the averages for the aluminum concentration are for five sets of determinations during the first period and three sets of determinations during the second period.

num remained in solution with the addition of 10 p.p.m. than with the addition of 5 p.p.m. During the latter part of the investigation the aluminum chloride solution used was prepared from the salt rather than from pure aluminum metal dissolved in strong hydrochloric acid. It was, therefore, less acid and since less sodium hydroxide was required to neutralize the acidity, less aluminum was precipitated as the silicate.

The phosphate concentration of the nutrient solutions remained near the original concentration during the earlier periods of growth. As the plants became older and absorbed more phosphorus, the concentration in the culture solutions was rapidly lowered. This is especially true in cultures 1 and 2, where the growth was best. In no case, however, did the phosphate concentration ever become lower than 0.5 p.p.m., a concentration found by Parker (13) to be sufficiently high for the maximum growth of corn.

*Effect of soluble aluminum on plant growth*

The injurious action of aluminum on the growth of the plants became evident at a very early stage of growth. With corn, which grew vigorously from the time it was placed in the nutrient solutions, injury was apparent in the roots after the third day in the aluminum solution, even where only 1 p.p.m. of aluminum was present. This injury was evidenced by a tendency toward stubbiness and a decrease in the number of lateral roots. The damage increased with increasing concentrations of aluminum. Soon the roots of the plants which were grown in solution cultures receiving aluminum became brownish in color, particularly the tips of the roots. The effects of aluminum on the tops were much slower in developing, becoming apparent only after the second week of growth. This injury was more evident in the size of the stalk than in the height of the plants and was more pronounced with increasing concentrations of aluminum.

The injuries to barley and sorghum were also evident at an early stage of growth with 1 p.p.m. of aluminum. As in the case of corn, the damage to the roots preceded decreased growth of tops, and the extent of injury increased with increasing concentrations of aluminum and with the age of the plants. The characteristic effects on sorghum are well shown in plate 1, figure 2. It will be noted that the injury from 1 p.p.m. of aluminum in culture 3C is very evident in both tops and roots. The leaves show a characteristic chlorosis similar to type-B chlorosis of corn described by Pettinger et al. (15). The roots, however, have the most pronounced lesions. They show very well the characteristic stubbiness, the absence of lateral roots, and the pronounced browning of the root tissue. In general, these characteristics agree with the descriptions given by other investigators (6, 8, 9, 11).

The corn plants were cut on October 10, whereas the barley and sorghum plants were cut on December 6. The appearance of the plants at harvest time is shown in plates 1 and 2; the yields of roots and tops are given in tables 2, 3, and 4.

It will first be noted from table 2 that the weight of corn in culture solutions without aluminum was only about one-half as much when grown at pH 4.5 as when grown at pH 6.0. The presence of 1 p.p.m. of aluminum in culture solutions of pH 4.5 reduced the yields about 25 per cent, whereas the presence of 2.28 and 4.56 p.p.m. reduced the yields 39 and 59 per cent respectively.

The yields of the sorghum plants at harvest time are given in table 3. It will be noted that the total yields in cultures without aluminum was about 70 per cent as high at pH 4.5 as at pH 6.0. When the nutrient solutions contained 1.04, 2.34, and 4.63 p.p.m. of aluminum, the yields at pH 4.5 were reduced 16, 82, and 87 per cent, respectively. The top-root ratio values, given in the last column of table 3, substantiate the conclusions obtained from observations on the appearance of the plants, namely, that the roots are relatively more injured than the tops by the presence of aluminum.



TABLE 2

*Average yields of corn in culture solutions of different aluminum and hydrogen-ion concentrations*  
(Average weights of two plants)

CULTURE NO.	pH OF SOLUTION	ALUMINUM		TOPS			ROOTS (OVEN-DRY)	TOTAL WEIGHT		TOP ROOT
		Added	Average amount in solution	Green weight	Oven-dry weight	Per cent dry matter		Absolute	Relative	
		p.p.m.	p.p.m.	gm.	gm.			gm.		
1. A, B, C . . . . .	6 0	0	0	304 1	42.7	14.4	5 5	48.2	198	7 8
2. A, B, C . . . . .	4 5	0	0 03	203 9	20 7	10.1	3 6	24.3	100	5 8
3. A, B, C . . . . .	4 5	1 0	1 03	148 9	16 2	10 9	2 2	18 4	75	7 4
4. A, B, C . . . . .	4 5	2 0	2 28	124.1	12 6	10 1	2 1	14 7	61	6 0
5. A, B, C . . . . .	4 5	5 0	4 56	87 5	8 6	9 8	1 3	9 9	41	6 6
6. A, B, C . . . . .	4 5	10 0	4 06	93 9	9 4	10 0	1 4	10 8	45	6.7

TABLE 3

*Average yields of sorghum in culture solutions of different aluminum and hydrogen-ion concentrations*

(Average weights of two plants)

POT NO.	pH OF SOLUTION	ALUMINUM		TOPS (OVEN-DRY)	ROOTS (OVEN-DRY)	TOTAL WEIGHT		TOP ROOT
		Added	Average amount in solution			Absolute	Relative	
		p.p.m.	p.p.m.			gm.		
1. A, B, C . . . . .	6 0	0	0	10 43	2.22	12 65	146	4 7
2. A, B, C . . . . .	4 5	0	0 02	6 97	1 70	8 67	100	4.1
3. A, B, C . . . . .	4 5	1 0	1 04	6 07	1 25	7 32	84	4 9
4. A, B, C . . . . .	4 5	2 0	2 34	1 42	0 18	1 60	18	7 9
5. A, B, C . . . . .	4 5	5 0	4 63	1 02	0 13	1 15	13	7 8
6. A, B, C . . . . .	4 5	10 0	5 41	0 72	0 08	0 80	9	9 0

TABLE 4

*Average yields of barley in culture solutions of different aluminum and hydrogen-ion concentrations*

(Average weights of two plants)

POT NO.	pH OF SOLUTION	ALUMINUM		TOPS (OVEN-DRY)	ROOTS (OVEN-DRY)	TOTAL WEIGHT		TOP ROOT
		Added	Average in solution			Absolute	Relative	
		p.p.m.	p.p.m.			gm.		
1. A, B, C . . . . .	6.0	0	0	15 92	1.53	17.45	105	9.6
2. A, B, C . . . . .	4.5	0	0 02	15.23	1 40	16 63	100	9.2
3. A, B, C . . . . .	4.5	1.0	1.04	6 15	0.45	6 60	40	13.6
4. A, B, C . . . . .	4.5	2.0	2.34	4.18	0.38	4.56	26	11.0
5. A, B, C . . . . .	4.5	5.0	4.63	4.27	0.37	4.64	28	11.6
6. A, B, C . . . . .	4.5	10.0	5.41	2 32	0.22	2.54	15	10 5

Table 4 and plate 2 show the results obtained with barley. It will be noted that in contrast to the results obtained with corn and sorghum, an increase in the hydrogen-ion concentration from pH 6.0 to 4.5 has reduced the yield of barley only very slightly. The presence of small amounts of aluminum, however, has had a marked deleterious effect. One part per million of aluminum has reduced the yields of tops 60 per cent and of roots 68 per cent, and the total yields in the presence of 2.34, 4.63, and 5.41 p.p.m. of aluminum have been reduced 74, 72, and 85 per cent, respectively. The relatively greater reduction in the yield of roots than of tops from the presence of aluminum has been much greater with barley than with corn, as will be seen from the top-root ratio values given in the last column.

#### GENERAL DISCUSSION

The data presented in this study show conclusively that relatively low concentrations of aluminum in culture solutions are toxic to the growth of plants. All three plants studied—corn, sorghum, and barley—were injured by 1 p.p.m. of aluminum in culture solutions at pH 4.5. At this concentration of aluminum, barley showed the greatest reduction in yield; corn, the next greatest; and sorghum, the least. The injury to the roots, however, was much more pronounced with sorghum than with corn, and at higher concentrations of aluminum, sorghum was injured considerably more than was corn. This order of injury is the same as that reported by McLean and Gilbert (9). The concentrations required to be injurious as found in this study, however, are considerably lower than has been reported by these investigations. This is especially true of corn. Thus McLean and Gilbert in their classification of plants as regards injury from aluminum place corn in the resistant group, the plants of which are "depressed by 14 p.p.m. of added aluminum or more." Barley is placed in the sensitive group of plants which are "depressed by 2 p.p.m. of added aluminum," whereas sorghum is considered mediumly sensitive, being "depressed by 7 p.p.m. of added aluminum or less." They give some data on the yield from cultures with and without aluminum which in some cases indicate toxicity at concentrations lower than those given in their classification. In a later investigation, however (10), these same authors found that the addition of 13.6 p.p.m. of aluminum resulted in yields of corn 93 to 96 per cent as high as where no aluminum had been added. They also found that rye, oats, alfalfa, buckwheat, onions, and redtop were stimulated by additions of 3.4 to 13.6 p.p.m. of aluminum in culture solutions. Moreover, the tap water used in these investigations contained 3 to 4 p.p.m. of aluminum. In this same investigation 5 p.p.m. of aluminum added as the citrate to nutrient solutions made with distilled water stimulated the growth of barley while 16 p.p.m. slightly depressed the growth. The addition of 5 p.p.m. of aluminum sulfate to nutrient cultures made up with tap water was slightly injurious to barley when small amounts of phosphate were present but gave no harmful effect with large amounts of phosphate. The addition of 16 p.p.m. aluminum depressed the yields of barley considerably.

The results of the investigations by McLean and Gilbert are reviewed in some detail here because they were conducted in such a way that there was no mutual precipitation of aluminum and phosphate; therefore they are not subject to one of the most serious criticisms of culture solution work with aluminum. Probably the chief criticism of these investigations is that one to six plants were grown in jars of only 250 cc. capacity. As a result, the total growth made by the plants even when no aluminum was added was very small. Moreover, the use of such small culture vessels must have resulted in a rapid change in the pH of the solutions. The pH values of certain cultures after plants had been in them for two days are given. Thus, with nutrient solutions having an original pH value of about 4.5, corn raised the pH value to 6.7; barley, to 6.4; but sorghum decreased the pH value to 3.9. It seems probable, therefore, that in some of the cultures not all of the aluminum added remained in solution. This would tend to explain why the minimum toxic concentrations found by these investigators were considerably higher than those reported in this study.

In a previous investigation by one of the writers (16) and later in a more comprehensive study in this laboratory (17) it was found that the concentrations of aluminum in acid soils were relatively low. As a result of the latter investigation it was concluded that in average field soils at pH 4.5 or above, the displaced soil solution usually contains less than 5 p.p.m. of aluminum, whereas soils of pH 5.0 seldom contain as much as 1 p.p.m. of aluminum in solution. Since it was found that a lowering of the salt concentration of the soil reduced the concentration of soluble aluminum at given pH values, it was concluded that in the presence of a crop or where frequent rains keep the salt content of the soil very low, the concentrations of aluminum in the displaced soil solution may not be more than 1 p.p.m. at pH 4.5, and still less at higher pH values. In view of these considerations and the relatively high concentrations of aluminum which had been found necessary by various investigators to produce toxicity in culture solutions, it was at first believed that the small concentrations of soluble aluminum present in acid soils could not have much effect on the growth of plants. In view of the results obtained in this investigation, however, it is apparent that this conclusion may not be warranted, since a concentration of 1 p.p.m. of aluminum was found injurious to all three crops studied and since it is quite likely that even lower concentrations would have been found injurious if they had been included in the study.

In applying results of culture solutions to soils it must be recognized, of course, that various factors may influence the concentrations of aluminum required to be toxic and, moreover, that these factors may be different under culture solution than under soil conditions. Thus some evidence was obtained by one of the writers (16) that the greater the percentage base saturation of soils the higher the concentration of aluminum required to be toxic. The injury from a given amount of aluminum is also believed by some investigators to be dependent at some extent on the composition of the nutrient solution

(1, 8, 18). Moreover, Spencer (19) in some work with *Rhododendron*, which has recently appeared in abstract form, concludes that "in general, the toxic action of aluminum decreases as the acidity of the solution increases."

A significant point that should be emphasized in applying the results of culture solutions to soil is that aluminum causes greater injury to the roots than to the tops of plants. In this investigation, for example, the top growth made by sorghum in the presence of 1 p.p.m. of aluminum was nearly as good as where no aluminum was present. The roots, however, were very badly injured (fig. 2). It appears, therefore, that in spite of their poor growth and severely injured condition, the roots were able to obtain enough nutrients from the solution to make a fair growth of top. Under soil conditions where the nutrients may not be as readily available, it is reasonable to suppose that a poor root system would be less able to absorb the necessary nutrients than in nutrient solutions, and consequently greater injury to the plant would result. This is indicated by the investigations of McLean and Gilbert (9) in which they found that plants injured by aluminum absorb dyes, nitrates, and water less readily than do normal plants.

It may also be expected that the injury from a given concentration of aluminum will be greater under unfavorable conditions of growth in soils than under more favorable conditions. In times of drought, for example, injury to plant growth from soluble aluminum might be greater than under conditions of normal rainfall for two reasons: (a) Low rainfall would mean little leaching and a high salt concentration in the soil and, therefore, a relatively high concentration of aluminum in the soil solution, and (b) plants injured by aluminum would suffer relatively more than normal plants from a lack of moisture, since they would not be able to utilize the moisture present as efficiently.

These considerations emphasize the need of determining the importance of various factors which may influence the concentrations of aluminum required to be toxic to various plants both in culture solutions and in soils.

#### SUMMARY

Corn, sorghum, and barley were grown in culture solutions with the purpose of determining the minimum concentration of soluble aluminum required to cause toxicity. The plants were alternated daily between complete nutrient solutions and similar solutions containing aluminum but no phosphate in order to prevent the precipitation of aluminum phosphate. Large culture vessels were used so as to make possible an accurate control of the aluminum, the phosphate, and the hydrogen-ion concentration of the nutrient solutions. The results obtained may be briefly summarized as follows:

Aluminum, present in the culture solutions in concentrations as low as 1 p.p.m., was definitely injurious to the growth of all three plants studied.

Increasing the concentration of aluminum above 1 p.p.m. progressively increased the injury to plant growth.

In the presence of 1 p.p.m. of aluminum, barley was most seriously injured. At higher

concentrations of aluminum, however, sorghum was injured more than barley, and corn was considerably less injured than either barley or sorghum.

The injurious action of aluminum was first noted in the roots and became more evident in both roots and tops with increasing age of the plants.

An increase in the hydrogen-ion concentration of the nutrient solution from pH 6.0 to 4.5 was most injurious to the growth of corn, less injurious to the growth of sorghum, and least injurious to the growth of barley.

These results show that aluminum may be injurious to plant growth in lower concentrations than have generally been considered necessary. It is pointed out that these concentrations of aluminum are within the range found in the displaced solution of acid soils of pH 4.5 to 5.0 and, therefore, that plant growth on such soils may be seriously injured from the presence of soluble aluminum.

Some of the factors which may influence the concentrations of aluminum required to be toxic both in culture solutions and in soils are briefly discussed.

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## PLATE 1

## EFFECT OF VARIOUS CONCENTRATIONS OF ALUMINUM ON GROWTH OF CORN AND SORGHUM

FIG. 1. Effect on corn.

Culture No. 1—pH 6.0.	No. Al
Culture No. 2—pH 4.5.	No. Al.
Culture No. 3—pH 4.5.	1.03 p.p.m. Al.
Culture No. 4—pH 4.5.	2.88 p.p.m. Al.
Culture No. 5—pH 4.5.	4.56 p.p.m. Al.
Culture No. 6—pH 4.5.	4.06 p.p.m. Al.

FIG. 2. Effect on sorghum

Culture No. 1C—pH 6.0.	No Al.
Culture No. 2C—pH 4.5.	No Al.
Culture No. 3C—pH 4.5.	1.04 p.p.m. Al.
Culture No. 4C—pH 4.5.	2.34 p.p.m. Al.
Culture No. 5C—pH 4.5.	4.63 p.p.m. Al.



FIG. 1



FIG. 2



## PLATE 2

## EFFECT OF VARIOUS CONCENTRATIONS OF ALUMINUM ON GROWTH OF BARLEY

Culture No. 1	pH 6.0	No Al
Culture No. 2	pH 4.5	No Al
Culture No. 3	pH 4.5	1.04 p.p.m. Al
Culture No. 4	pH 4.5	2.34 p.p.m. Al
Culture No. 5	pH 4.5	4.63 p.p.m. Al
Culture No. 6	pH 4.5	5.41 p.p.m. Al





# THE INFLUENCE OF MOISTURE UPON THE RAPIDITY OF DECOMPOSITION OF LOWMOOR PEAT<sup>1</sup>

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The problem of the rapidity of decomposition of peat has certain important theoretical aspects and practical applications. Different types of peat, such as the lowmoor, forest, highmoor, and sedimentary, vary considerably in their chemical composition, in the nature of the microbial flora inhabiting these peats, and in the rapidity with which they can be attacked by microorganisms; it is, therefore, natural to expect that these peats will also vary considerably in the rapidity of their decomposition, as influenced by different environmental conditions and treatment, and in the extent of liberation of the plant nutrients in an available form.

One may use for an investigation of this nature a typical lowmoor peat, which contains about 3 per cent nitrogen or 20 per cent organic nitrogenous substances, about 5 to 10 per cent mineral constituents, about 50 per cent lignins and lignin-like complexes, about 5 to 8 per cent hemicelluloses, and a number of other substances in lower concentrations, such as fats, waxes, and cellulose. This type of peat usually has a pH of 5.0 to 6.5 and contains an abundant flora of microorganisms (2), so that as soon as it is drained, it begins to undergo rapid decomposition, without further treatment. Theoretically, it is important to determine what chemical constituents of the peat are attacked first, under these conditions, how rapidly the nitrogen becomes available, and how the conditions of decomposition affect the rate of decomposition. Practically, especially if such types of peat are not more than 2 to 3 meters deep when drained, it is essential to establish how long such peat can be cultivated and how rapidly it will tend to disappear completely, as a result of continuous drainage and cultivation, leaving only a clay, sand, or rock bottom.

Results obtained in the study of decomposition of lowmoor peat under controlled conditions need not necessarily apply to the decomposition of other types of peat, especially of highmoor peat, since the depth, chemical composition, physico-chemical nature, and microbiological population of the latter is

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so distinctly different from that of other types of peat that the results will be markedly different.

Among the conditions which have a decided influence upon the decomposition of a lowmoor peat is the moisture content. This is especially true for warm regions, where the favorable annual temperature does not serve as a check upon the activities of microorganisms. It is well established that the anaerobic conditions of the peat bog, resulting from the saturation of the bog with water, are largely responsible for the formation and accumulation of the peat. It is also known that when a region is subject to a period of dryness, the surface layers of the peat bog will undergo rapid decomposition, as shown by the formation of the limiting horizon (Grenzhorizont) in the European highmoor peats. It still remains to be determined experimentally how the relative abundance of moisture will affect the rate of decomposition and whether, by

TABLE 1  
*Influence of moisture content upon the decomposition of a lowmoor peat, from Florida*

MOISTURE CONTENT	WEIGHT OF ORIGINAL PEAT*	INCUBATION	CARBON DIOXIDE LIBERATION	NITROGEN LIBERATION		
				NH <sub>4</sub> -N	NO <sub>3</sub> -N	Total N
<i>per cent</i>	<i>gm.</i>	<i>days</i>	<i>mgm. C</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm</i>
78	100	54	81 0	.	6 56	.
55	100	54	196.2	.	3 84	.
40	100	54	89 0	.	1 95	.
28	100	54	29 4	.	.	.
78	300	58	236 9	4.8	16 52	21 32
55	300	58	822 5	40 2	4 50	44 70
78	300	79	.	13 2	17 17	30 37
55	300	79	.	60 0	8 01	68 01

\* The moisture content of the original peat was 78 per cent.

controlling the moisture content, one may be able to control also the speed of decomposition of the peat.

The experiments reported in this study were undertaken with the idea of obtaining further information on the problem of the influence of environmental conditions upon the rate of peat decomposition. The first experiment was carried out with peat (largely *Cladium*) obtained from the surface 30 cm. of the Fellsmere peat area in Florida. Fresh moist peat in 100-gm. and 300-gm. portions was adjusted to different moisture contents, by gradually being dried down at room temperature; the preparations were then placed in flasks, connected with the respiration apparatus and incubated at 28°C. Air, freed from CO<sub>2</sub>, was passed over the surface of the peat and the CO<sub>2</sub> liberated was absorbed in standard Ba(OH)<sub>2</sub> solution. At the end of different periods of incubation, the ammonia and nitrate contents of the peat were determined. The results given in table 1 show that by reducing the moisture content to 50 or 60 per cent of the total peat, there is a rapid increase in the rate of decom-

position; from 2.5 to 3.5 times as much carbon was given off as  $\text{CO}_2$  from the same amount of peat when the moisture was reduced from 78 to 55 per cent. It should be mentioned here that the particular peat material was taken from a layer above the water table; this peat was, therefore, partly drained, as shown by its comparatively low original moisture content, namely 78 per cent, instead of about 90 per cent moisture, which is ordinarily found in this type of peat in the undrained bog. Parallel with the liberation of the carbon as  $\text{CO}_2$ , nitrogen was liberated as ammonia and nitrate. It is of interest to note that the lower the moisture content the greater was the relative liberation and accumulation of ammonia over the nitrate. When the moisture content was reduced further, the rate of decomposition was diminished, so that, at 40 per cent moisture, the rate was about the same as at 78 per cent. At 28 per cent moisture, decomposition was markedly reduced, since the peat now became almost air-dry.

In order to study this problem in greater detail, quantities of peat were obtained from the same area, but at different depths. The peat was now taken from a cultivated field, 75 feet from a drainage sublateral, and midway between two field ditches, 40 feet apart and 18 inches deep. The layers from which the peat samples were removed were marked as follows: A, at a depth of 0-30 cm., B, at 30 to 60 cm., and C, at 60 to 75 cm. depth. The second sample was just below the surface of the water table, and the third sample was 30-45 cm. below the surface of the water table. The moisture content of A, or surface sample of peat, was 81.2 per cent, of B, or the second layer, 89.84 per cent, and of C, the third layer, 89.95 per cent; the last two layers were completely saturated with water. The peat was taken in such a manner so as to disturb it as little as possible. This was done by pressing down heavy, tin cans, 15 cm. long and about 4 cm. in diameter, with small holes in the bottom, at the particular depths of peat; the cans were then removed with the peat, closed with tight fitting tops and immediately sealed; the small holes at the other end were also closed, and the cans shipped to the laboratory. The peat was immediately removed from the cans and used for the following experiment.

Several 500-gm. quantities of the fresh peat were weighed out; some were left with the original moisture, while some were dried down, at about  $50^\circ\text{C}.$ , so as to reduce the moisture of the peat, in order to obtain a varying water content. The peat samples, with the moisture thus adjusted, were all inoculated with a suspension of fresh garden soil, placed in liter flasks with side arms, and connected with the respiration apparatus in the incubator ( $28^\circ\text{C}.$ ). Carbon-dioxide-free air was passed over the peat and collected in standard barium hydroxide solution. The plan was to allow the peat to decompose under definite conditions for a period of 18 to 24 months. However, since as a result of decomposition, there is an accumulation of salts, which, under natural conditions, are removed by drainage or by growing plants, the accumulated salts and soluble organic substances were leached out at definite intervals of

time, so as to prevent any possible injury that they may have upon the continuous decomposition of the peat. At the end of the first and second 6-month periods, the peat samples were removed, the moisture content was determined, and all the peats were leached with distilled water. The leachings were analysed for ammonia, nitrate, total nitrogen, total organic matter, and bases. The peat samples were again adjusted to the original moisture content, again inoculated with a fresh soil suspension, and returned to their respective flasks.

At the end of 560 days' decomposition, the experiment was discontinued. The peat samples were removed and analyzed. A comparison of the composition of the peat at the beginning and at the end of the decomposition periods,

TABLE 2  
*Decomposition of peat at different moisture contents, at constant temperature, for a period of 560 days*

On basis of original 100 gm. of dry peat

PEAT LAYER DEPTH	MOISTURE CONTENT	TOTAL DRY MATERIAL AT START	TOTAL DRY MATERIAL AT END OF DECOMPOSITION	LOSS OF MATERIAL, DUE TO DECOMPOSITION	ORGANIC MATTER LIBERATED AS CO <sub>2</sub> (C × 1.8)	ORGANIC MATTER REMOVED IN TWO LEACHINGS	LOSS OF ORGANIC MATTER UNACCOUNTED FOR*
cm.	per cent	gm.	gm.	per cent	per cent	per cent	per cent
0-30	83.8	100	90.4	9.6	4.78	1.50	3.32
0-30	79.9	100	90.2	9.8	5.38	1.38	3.04
0-30	71.3	100	79.9	20.1	10.86	3.84	5.40
0-30	52.8	100	80.7	19.3	10.95	3.87	4.48
0-30	33.3	100	87.4	12.6	4.31	1.58	6.71
30-60	89.8	100	89.4	10.6	6.58	2.09	1.93
30-60	73.5	100	85.8	14.2	11.95	2.89	-0.64
30-60	69.5	100	83.6	16.4	14.45	2.95	-1.00
30-60	58.8	100	84.9	15.1	13.49	3.03	-1.52
60-75	90.9	100	91.2	8.8	6.55	1.98	0.27
60-75	81.3	100	75.4	24.6	18.29	3.12	3.19
60-75	72.8	100	81.1	18.9	17.35	2.98	-1.43
60-75	63.0	100	87.9	12.1	9.43	2.02	0.65

\* This loss is partly accounted for by the inorganic constituents of the leachings (table 4) which were not included in these results; — indicates an excess.

as well as of the extent and nature of decomposition at the different moisture contents and of the rate of decomposition of peat taken from different depths, permits one to draw some very interesting conclusions. In order to facilitate comparisons, the results were all recalculated on the basis of 100 gm. of original dry peat material.

Table 2 gives the total loss in weight as a result of decomposition, the amount of organic matter decomposed and liberated as CO<sub>2</sub> (by multiplying the carbon of the CO<sub>2</sub> by 1.8), and the amount of organic matter made soluble and removed in the leachings after 6 and 12 months' decomposition. These results show that at certain moisture contents, namely at about 50 to 80 per cent, the decomposi-

tion of the peat is at a maximum. As much as 20 per cent of the total peat, and in one instance nearly 25 per cent of the peat, was decomposed in less than 19 months, under optimum moisture and temperature conditions. Most of the peat decomposed was lost into the atmosphere as carbon dioxide. Actually, on the average of the seven samples of peat, which had decomposed under optimum moisture conditions, about 14 per cent of the total peat was liberated as  $\text{CO}_2$  into the atmosphere. By accounting for the moisture and the ash contents of the peat the percentage decomposition will be found to be still greater.

TABLE 3

*Changes in nitrogen and ash content and in reaction of a lowmoor peat, as a result of continuous decomposition*

On per cent basis of dry peat

DEPTH OF PEAT	MOISTURE CONTENT	NITROGEN CONTENT		ASH CONTENT		REACTION	
		At start	After 560 days decom- position*	At start	After 560 days decom- position*	At start	After 560 days decom- position*
<i>cm.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pH</i>	<i>pH</i>
0-30	83.8	3.03	3.30	9.15	10.56	6.0	5.3
0-30	79.9	3.03	3.43	9.15	10.05	6.0	5.0
0-30	71.3	3.03	3.35	9.15	9.40	6.0	4.6
0-30	52.8	3.03	3.53	9.15	10.00	6.0	4.6
0-30	33.3	3.03	3.06	9.15	10.00	6.0	5.3
30-60	89.8	3.24	3.41	5.10	5.16	5.5	4.7?
30-60	73.5	3.24	3.77	5.10	5.90	5.5	5.4
30-60	69.5	3.24	3.66	5.10	5.73	5.5	5.5
30-60	58.8	3.24	3.55	5.10	5.95	5.5	5.5
60-75	90.9	2.97	3.73	5.02	5.27	6.0	5.5
60-75	81.3	2.97	4.03	5.02	6.87	6.0	5.0
60-75	72.8	2.97	3.76	5.02	6.65	6.0	5.1
60-75	63.0	2.97	3.46	5.02	5.83	6.0	5.6

\* Allowance must be made for the nitrogen and ash removed in the two leachings, as shown in table 4.

There is no doubt that when the peat is brought into the laboratory artificial conditions are created which are totally different from those prevailing in the field. However, the general principles will be found to be alike in both instances.

Table 3 shows the changes in the nitrogen, in the ash content, and in the reaction of the peat as a result of the continuous decomposition, under standard conditions. As a result of decomposition, there was a marked increase in the relative amount of nitrogen and ash. By taking into consideration the quantities of nitrogen and minerals removed in the two leachings (table 4), one can readily calculate that the greatest increases in the liberation and accumulation of nitrogen and ash corresponded to the most active decomposition of the peat. The pH values decreased, or the acidity increased, with an increase in the



decomposition, no doubt as a result of the formation of nitrates from the ammonia which resulted from the decomposition of the nitrogenous complexes of the peat.

On computing the ratio between the carbon liberated as  $\text{CO}_2$  to the nitrogen liberated as ammonia and nitrate, it is found to be narrowest with the highest moisture content. The average ratio for all the peats is  $\text{C/N} = 20.5$ . If one considers the fact that the carbon-nitrogen ratio in the peat itself is about 18, and further the fact that this peat has only a low content of available carbohydrates (cellulose, hemicelluloses), as seen from the complete analysis of

TABLE 4

*Influence of moisture content of peat on the rapidity of its decomposition, as measured by the forms of nitrogen liberated in an available form and composition of leachings*

On the basis of 100 gm. of original dry peat

DEPTH OF PEAT	MOISTURE CONTENT	TOTAL NITROGEN LIBERATED*	COMPOSITION OF LEACHINGS AFTER 6 AND 12 MONTHS DECOMPOSITION			C/N†
			Total water- soluble matter	Total nitrogen	Ash	
cm.	per cent	mgm	mgm	mgm	mgm.	
0-30	83.8	150.3	1,398	57.8	623	17.7
0-30	79.9	130.6	1,383	69.2	643	22.9
0-30	71.3	291.3	3,843	235.3	1,045	20.7
0-30	52.8	279.9	3,872	258.3	1,053	21.7
0-30	33.3	122.2	1,583	95.3	571	19.7
30-60	89.8	208.0	2,088	115.8	1,009	17.6
30-60	73.5	356.3	2,895	171.6	748	18.7
30-60	69.5	362.0	2,954	207.5	726	22.2
30-60	58.8	299.5	3,054	155.0	886	25.0
60-75	90.9	264.4	1,976	60.0	1,000	13.8
60-75	81.3	489.2	3,122	152.6		20.8
60-75	72.8	410.5	2,982	149.1		23.4
60-75	63.0	231.5	2,023	154.3		22.6
Average ..						20.5

\* As  $\text{NH}_3$  and  $\text{NO}_3\text{-N}$  in the two leachings and at the final analysis.

† C = carbon liberated as  $\text{CO}_2$ ; N = nitrogen liberated as  $\text{NH}_3$  and  $\text{NO}_3$ .

the peat, one must be led to conclude that the major organic complexes, namely, the lignins and lignin-like complexes and the proteins underwent active decomposition. In the case of fresh plant residues, the contrary is true, because of the fact that these residues contain a variety of substances, some of which decompose readily, while others are resistant to decomposition. This type of peat, however, is made up largely of two organic chemical complexes (table 5), namely, the lignins and proteins, which probably exist in the peat in chemical combination; one would expect, therefore, that there should be a parallel liberation of the carbon and the nitrogen, when this peat undergoes decomposition.

The fact that the ratio of carbon to nitrogen in the decomposition products is somewhat wider than in the peat itself would tend to indicate that the residual material gradually becomes enriched in nitrogen; this was actually found to be the case, as shown in table 3. The greater the extent of decomposition of the peat, the higher is the nitrogen content of the undecomposed material.

In order to compare further the influence of the moisture content of peat, as well as the depth from which the peat was taken, upon the rate of its decomposition, one should compare the rate of decomposition, as expressed best by the curves giving the course of evolution of CO<sub>2</sub> (figs. 1, 2, 3). In figure 1, the data for the surface layer of peat are given. The results clearly point to the fact that the greatest decomposition of this type of peat takes place at a moisture content of 53 to 71 per cent; when the moisture drops much below 50

TABLE 5  
*Proximate composition of peat taken at three different depths*  
Per cent basis of dry material

	DEPTH OF PEAT, CM		
	0-30	30 60	60-75
Chemical constituents:			
Ether-soluble	1 06	1 46	1 57
Cold and hot water-soluble	4 07	4 77	4 23
Alcohol-soluble	1 97	1 95	2 04
Hemicelluloses	3 03	2 81	4 10
Cellulose	Tr.	Tr.	Tr.
Lignin	50 60	51 39	48 42
Crude protein	18 94	20 25	18 56
Ash	9 15	5 10	5 02
Total	88 82	87 73	83.94

per cent of the total peat or is increased to 80 per cent or above, decomposition is materially arrested. In the case of the second layer (fig. 2), similar results were obtained, except that none of the samples were dried sufficiently to reduce the moisture below 59 per cent; however, at 90 per cent moisture, decomposition was markedly diminished. The results obtained on the decomposition of the deepest layer are similar to those found for the material taken from the surface layer; at 73 to 81 per cent moisture, decomposition was at an optimum, dropping rapidly, in course of time, with the higher or lower moisture contents.

In order to compare the rates of decomposition of the peat taken from the three different layers, the data for each peat were averaged and the averages plotted in figure 4. The results show that, during the first 8 months, the peat taken from the surface layer (partly drained) decomposed most rapidly; however, after 12 months of decomposition, the peat taken from the deepest layer

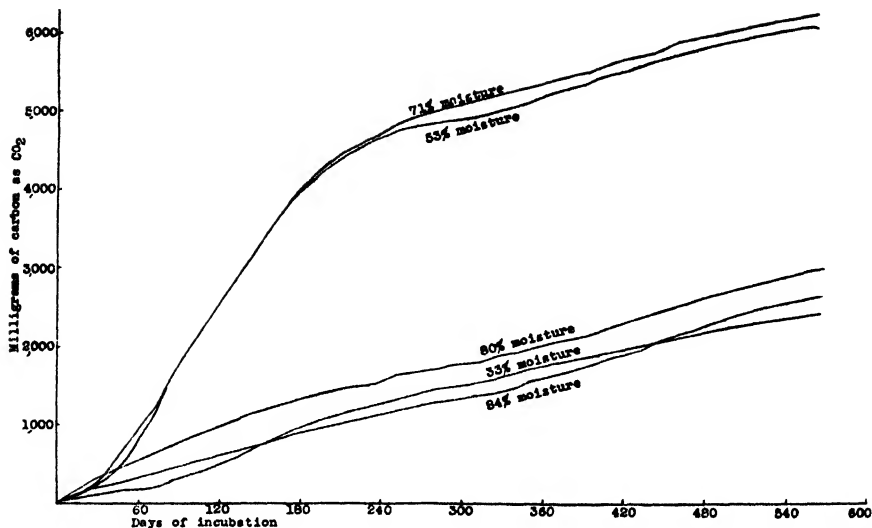


FIG. 1. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO<sub>2</sub> IN THE DECOMPOSITION OF PEAT TAKEN FROM THE SURFACE 30 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

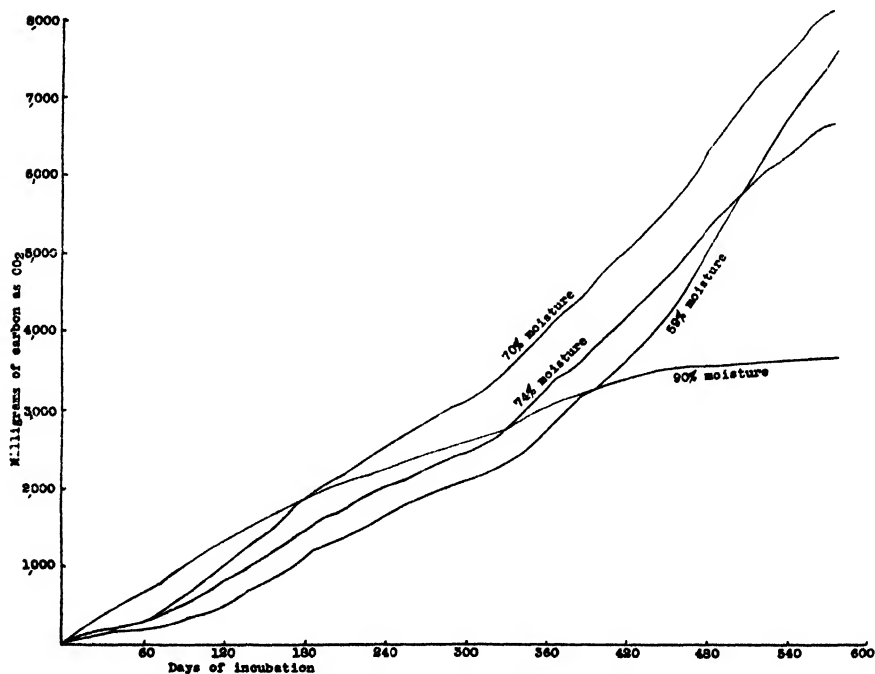


FIG. 2. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO<sub>2</sub> IN THE DECOMPOSITION OF PEAT TAKEN FROM THE 30-60 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

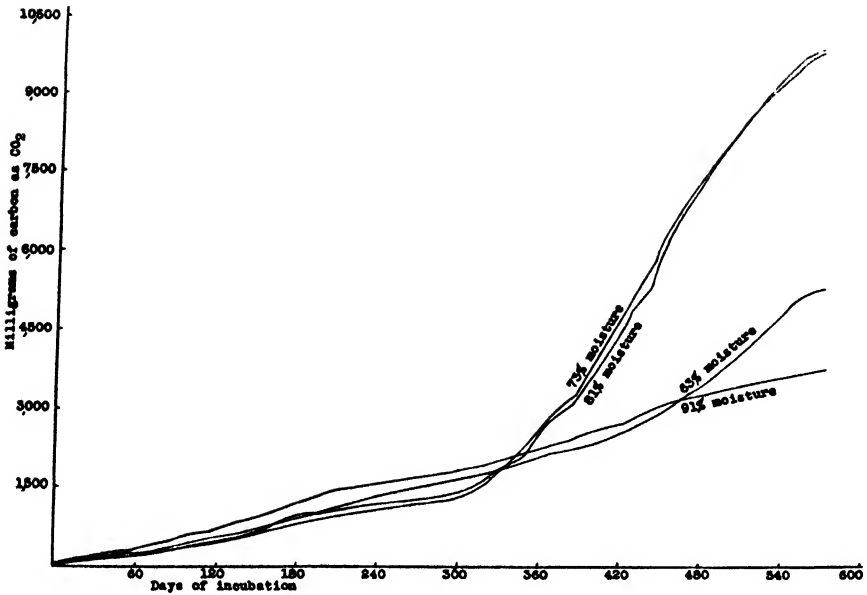


FIG. 3. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO<sub>2</sub> IN THE DECOMPOSITION OF PEAT TAKEN FROM THE 60-75 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

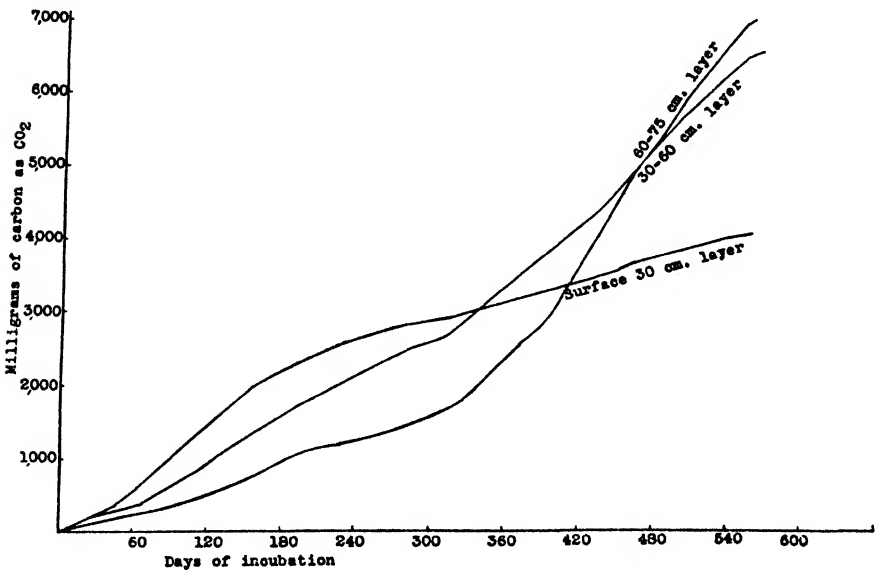


FIG. 4. COMPARATIVE RATE OF EVOLUTION OF CO<sub>2</sub> IN THE DECOMPOSITION OF A LOWMOOR FLORIDA PEAT, TAKEN FROM THREE DIFFERENT DEPTHS, ON THE BASIS OF 100 GM. OF DRY PEAT

underwent most active decomposition, with the middle layer coming between. The following explanation for this difference suggests itself: peat saturated for many centuries with water is in a certain reduced state, with only anaerobic bacteria finding conditions favorable for their growth and reproduction. When this peat is drained and brought in contact with atmospheric oxygen, a certain "lag period" has to pass, before the aerobic organisms are able to decompose the peat constituents actively. Whether this lag period consists in the destruction of certain substances toxic to aerobic organisms or whether it consists in the oxidation of the substances present in a reduced condition, remains to be

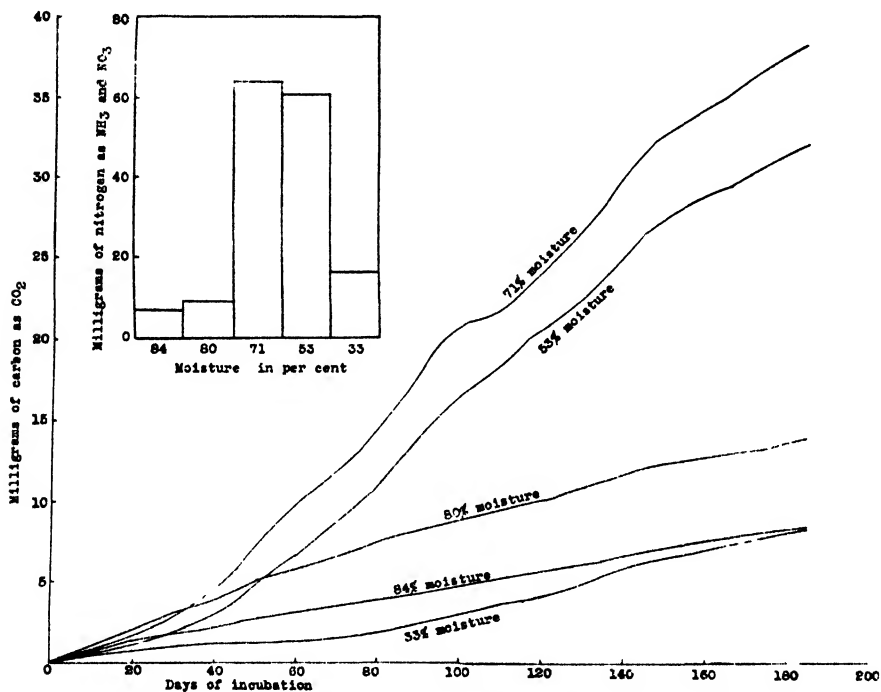


FIG. 5. INFLUENCE OF MOISTURE UPON THE DECOMPOSITION OF PEAT, TAKEN FROM THE SURFACE 30 CM. OF THE BOG, DURING THE EARLY STAGES OF DECOMPOSITION, AS SHOWN BY EVOLUTION OF CO<sub>2</sub> AND LIBERATION OF NITROGEN IN AN AVAILABLE FORM

determined. It may possibly be due to a gradual adaptation of the aerobic organisms to the resistant organic constituents of the peat, especially the lignins and the proteins, which were accumulated because of their resistance to attack by anaerobic bacteria.

The results presented in figure 5 illustrate the relative liberation of carbon as CO<sub>2</sub> and of nitrogen as ammonia and nitrate from the surface peat, during the early stages of decomposition. The two are found to run parallel, as brought out in the tables, while the influence of moisture upon the decomposition of the peat is especially emphasized.

TABLE 6  
*Proximate chemical composition of peat after 560 days' decomposition under different moisture conditions*  
Per cent of dry material

[illegible]

At the end of the decomposition period, the residual peat was dried and subjected to a proximate analysis. The results given in table 6 show that the various chemical constituents of the peat did not decompose with the same rate. The ether-soluble substances, which make up only a small part of the low moor peat, tended to be low with the low moisture and high with the higher moisture, whereas the alcohol-soluble substances tended to increase with conditions more favorable to decomposition. The hemicelluloses did not change appreciably in concentration, but the concentration of the lignins and proteins tended to increase. The general conclusion from these analyses is that the composition of the peat was in a condition of relative equilibrium, in

TABLE 7  
*Alkali solubility of peat materials*  
Per cent of dry peat

DEPTH	KEPT AT A MOISTURE CONTENT OF	SOLUBLE IN COLD 2 PER CENT $\text{NH}_4\text{OH}$	SOLUBLE IN HOT 4 PER CENT $\text{NaOH}$ AND PRECIPITATED BY $\text{HCl}$	TOTAL ALKALI SOLUBLE
<i>cm.</i>	<i>per cent</i>			
0-30	Control	30 0	24 3	54.3
0-30	83 8	22 4	35.8	58.2
0-30	79.9	19 8	35.7	55.5
0-30	71 3	39 5	26 1	65.6
0-30	52 8	37 6	25 6	63 2
0-30	33 3	25 6	34.2	59.8
30-60	Control	15 8	35 6	51.4
30-60	89.8	11 8	40 0	51 8
30-60	73.5	21.7	34 8	56 5
30-60	69.5	21 0	34 6	55 6
30-60	58.8	22 9	31.1	54 0
60-75	Control	25.6	26.4	52.0
60-75	90.9	9.5	47.8	57 3
60-75	81.3	35 4	30 6	66 0
60-75	72 8	28 8	31 2	60 0
60-75	63 0	15 2	38 3	53 5

which the various constituents decompose alike; here again the decomposition of the peat is quite different from that of fresh plant residues.

In order to determine whether any differences are found in the so-called "humus" fractions of the peat, the fresh and decomposed samples of peat were first treated with dilute  $\text{HCl}$  to remove the bases. Definite amounts of the samples thus treated were washed and dried and extracted with cold 2 per cent  $\text{NH}_4\text{OH}$  solution. The extract was filtered off, evaporated, dried to constant weight, and ignited. The residues from the ammonia extraction were treated with 4 per cent  $\text{NaOH}$  solution at 15 pounds pressure for 1 hour; the filtered extracts were neutralized with  $\text{HCl}$  and the precipitates formed were removed, washed, dried, and weighed (table 7). The results show that under conditions

of optimum decomposition there is an increase in the alkali-soluble fractions, especially the one soluble in cold dilute ammonium hydroxide (*matière noire* of Grandeau). It is doubtful whether this is merely a stage in the decomposition; it is probably more a result of the decomposition of the resistant lignin and protein complexes, as found to be the case in the decomposition of manure composts (1).

One need not, however, expect that the results obtained from the foregoing investigations on the decomposition of the lowmoor peat from Florida would hold exactly true for other peats or even for other lowmoor peats. This can well be illustrated by reporting the results of another experiment on the influence of drying of peat upon its decomposition; for this purpose, a lowmoor (*Carex*) peat from Newton, N. J., was used (3). The previous peat, from Florida, was taken fresh from the field, so that it had very little chance to decompose aerobically, except in the surface layer, before the experiment was

TABLE 8  
*Influence of moisture upon the rapidity of decomposition of a lowmoor peat from Newton, New Jersey*  
On basis of 20 gm. of dry peat

TREATMENT OF PEAT	MOISTURE CONTENT	CO <sub>2</sub> LIBERATED IN 21 DAYS	NITROGEN LIBERATED IN 21 DAYS		
			NH <sub>3</sub> -N	NO <sub>3</sub> -N	Total N
	<i>per cent</i>	<i>mgm C</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Untreated, 60 gm	66 6	16 4	1 6	8 2	9 8
Air-dried,* 23 gm., + 20 cc water.	53 5	115 1	25 1	3 8	28 9
Air-dried,* 23 gm., + 37 cc. water† .	66 7	41 9	9 6	5 6	15.2
Air-dry peat, 11.5 gm., + 30 gm. moist peat	51 8	109 4	22 4	4 2	26.6

\* Only 15 per cent moisture left.

† Saturated with water, not well absorbed; anaerobic environment.

started. The Newton peat was well decomposed in a pile, probably for a year or so, before it was used for the following experiment. The peat, when taken from the pile, contained 33.3 per cent dry matter. Sixty-gram portions of this peat were used for the decomposition studies. Some of the portions were left untreated, whereas others were thoroughly air-dried in the laboratory, so that the moisture was reduced to 15 per cent of the peat. When the same amount of distilled water was added to replace the loss, it was not well absorbed and the peat looked saturated with water. Only enough water was taken up by the peat to bring it to 53.5 per cent moisture.

The results given in table 8 show that the air-dried peat remoistened decomposed much more rapidly than the original peat remaining moist. However, the rate of decomposition of the air-dried peat was markedly reduced if an excess of water was used. The ratio between the carbon liberated as CO<sub>2</sub> and the nitrogen liberated as ammonia was considerably narrower in the case of this peat than in the case of the Florida peat. This is due not so much to the



difference in the chemical composition of the two peats, as to the fact that the Newton peat was allowed to compost for a long time, exposed to air and rain-fall, whereas the Florida peat was freshly taken from the bog.

#### SUMMARY

1. The moisture content of peat was found to have a marked influence upon the rate of its decomposition.

2. Under optimum moisture conditions, about 15 per cent of the total dry material of lowmoor peat was decomposed in 18 months, as indicated by the volatile products liberated.

3. By controlling the amount of water in the peat, one can almost at will control the speed of peat decomposition.

4. The optimum moisture content for the decomposition of a lowmoor peat was found to be at 50 to 80 per cent of the total moist peat; above and below that optimum, the rate of peat decomposition rapidly diminishes.

5. The lowmoor peat used in these experiments was in a condition of chemical equilibrium, and decomposed as a whole, without any chemical complexes disappearing more rapidly than others. However, there was a certain tendency for a somewhat more rapid decomposition of the non-nitrogenous than the nitrogenous complexes, which led to an increase in the nitrogen content of the residual peat.

6. In the process of decomposition of a lowmoor peat, there was a parallel liberation of carbon as  $\text{CO}_2$  and of nitrogen as ammonia, with an average ratio of C/N liberated as 20:1. This is somewhat wider than the C/N ratio of the peat itself, which is about 18:1, and tends to explain the increasing nitrogen content of the peat as a result of decomposition.

7. As a result of the decomposition of the peat there was an increase in the complexes soluble in dilute alkali solutions.

8. Drying of peat and then remoistening it greatly stimulated its decomposition.

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# THE INFLUENCE OF TEMPERATURE ON THE NITRATE CONTENT OF SOIL IN THE PRESENCE OF DECOMPOSING CELLULOSE<sup>1</sup>

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It is commonly known that the decomposition of cellulose in soil results in the depletion of soil nitrates. In certain sections of the country farmers have learned that in order to avoid impoverishment of the soil they must remove straw from the field instead of plowing the straw into the soil. The micro-organisms engaged in decomposing cellulose utilize the soil nitrate. Consequently, unless a soil is rich in organic nitrogen which can be converted readily into nitrate, nitrogen starvation of crops results. In addition to an adequate supply of organic nitrogen in the soil, suitable conditions of temperature, moisture, and soil reaction must obtain if nitrification is to proceed satisfactorily.

Among the investigations dealing with the depletion of soil nitrates as a result of cellulose decomposition in soil, those of Sievers and Holtz (13), Collison and Conn (3), and Allison (1) may be mentioned. These publications contain reviews of the literature on the subject.

Most of the studies concerning the effect of temperature upon the nitrate content of the soil during the process of cellulose decomposition have been devoted to the influence of seasons and have been carried out in the field. Batham and Nigam (2), Jenny (6), Owen and Denson (10), and Panganiban (11) have reported studies along this line. Russell, Jones, and Bahrt (12) investigated the influence of varying temperatures on the process of nitrification, but did not consider the effect of cellulose.

The present study was suggested by results obtained by Jones (7) in the use of paper pots for the growth of plants in the greenhouse. The foliage of the plants turned yellow. When some of the plants were removed from the pots and examined it was found that the roots had grown toward the periphery of the ball of soil and were in contact with the water-soaked paper of the pots. This led to the assumption that the microorganisms which were attacking the paper of the pots had utilized the nitrate of the soil. When readily available nitrogen was supplied to the plants they regained their normal color, demonstrating that the yellowing was due to nitrogen starvation.

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It was further observed that the degree and rapidity of the yellowing of the plants seemed to vary with seasons. This suggested the possibility that some seasonal factor may have been responsible for the variation. Since the amount of moisture added to the pots was uniform, it appeared probable that temperature was the factor which influenced the degree and severity of the yellowing of the plant foliage.

The experiments reported in this paper were planned to investigate the influence of temperature on the depletion of soil nitrates as a consequence of cellulose decomposition in the soil.

#### EXPERIMENTAL

The experimental work was divided into two parts. Part I consisted of a series of four experiments in which fallow soil was employed. Periodic nitrate

TABLE I  
*The plan of the experiments*

	EXPERIMENT I	EXPERIMENT II	EXPERIMENT III	EXPERIMENT IV
Soil treatment..	Soil untreated  Soil + cellulose	Soil untreated Soil + CaCO <sub>3</sub> Soil + CaSO <sub>4</sub> Soil + cellulose Soil + cellulose + CaCO <sub>3</sub> Soil + cellulose + CaSO <sub>4</sub>	Soil untreated  Soil + cellulose	Soil untreated Soil + CaCO <sub>3</sub>  Soil + cellulose Soil + cellulose + CaCO <sub>3</sub>
Incubating temperature, °C...	10, 15, 20, 25, 30, 35	15, 25, 35	10, 15, 20, 25, 30, 35	10, 15, 20, 25, 30, 35
Time of incubation.....	7 weeks	7 weeks	7 weeks	7 weeks

determinations were made from the soil of the various pots to indicate the influence of decomposing cellulose on the nitrate content of the soil. Part II consisted of four experiments in which the soil employed, its treatment, and the incubating temperatures were identical in each instance with those of the first section. Tomato plants were grown in the pots of the second section, the dry weight of the plants at the end of the growing period being taken as an indication of the nitrate available in the soil. The two parts of the experimental work are considered separately in the following pages. The plan of the experiments is shown in table 1.

The soil employed in this investigation was a light, sandy loam common in the valley of the Connecticut River. The compost used in the third experiment was made from the same type of soil. All soil was air dried and screened to give it uniformity and to remove stones, roots, and other debris. Cellulose and other materials were carefully mixed with the soil before it was put into the

pots. Into each pot  $5\frac{1}{2}$  kgm. of soil were put. Water was added and maintained at 60 per cent of saturation. Metal pots were used which had no provision for drainage. Soil temperature was maintained by setting the pots into a constant soil-temperature apparatus which is illustrated in plate 1.

Incubation was carried on for 7 weeks for each experiment, samples of the soil for nitrate determination being taken each week. The samples were of sufficient quantity to yield 100 gm. of air-dried soil from each pot. The soil was dried at about  $25^{\circ}\text{C}$ ., and screened through a 20-mesh sieve. The nitrate determinations were made by reduction method mentioned by Fred and Waksman (4), which employs Devarda's Alloy as a reducing agent. The technic is as follows:

Extract 100 gm. of soil with 500 cc. of distilled water. (The extraction was accomplished by vigorous shaking for 10 minutes in a mechanical shaker.) Filter through filter paper. Collect 250 cc. of the filtrate in a Kjeldahl flask. Add 5 cc. of a 50 per cent solution of sodium hydroxide and distil until 150 cc. have been driven over. The distillate contains the free ammonia of the soil and is discarded. Make the contents of the flask up to the original volume with distilled water and allow to cool to room temperature. Add 2 gm. of Devarda's alloy and distil slowly until 150 cc. have been driven over into a measured quantity of  $N/14$  sulfuric acid. After distillation is completed, titrate the sulfuric acid with  $N/14$  sodium hydroxide, using Congo red as an indicator. The results are obtained as milligrams of nitrogen in 50 gm. of soil, and are calculated to parts per million of nitrate.

The reduction method was chosen in preference to a colorimetric method because with the former a direct nitrate determination can be made on a much larger quantity of soil than with the latter. For purposes of comparison a number of the samples were also analyzed for nitrate content with a colorimetric method which employs phenoldisulfonic acid. The results obtained with the reduction method compared satisfactorily with those obtained with the colorimetric method, the former being somewhat more uniform for a series of samples from the same soil.

In experiments in which lime was employed the pH value of the soil was determined electrometrically at the end of the incubation period, a quinhydrone electrode being used.

#### PART I. INFLUENCE OF ADDED CELLULOSE ON SOIL NITRATE AS INDICATED BY THE NITRATE DETERMINATIONS FROM THE SOILS

##### *Experiment 1*

The soil chosen for this experiment was low in nitrate as well as in total nitrogen. There were 50 ppm. of nitrates and less than 1 mgm. of total nitrogen per gram of soil. The soil had not been previously limed nor treated with fertilizer. Six temperatures were used for incubation, from  $10^{\circ}$  to  $35^{\circ}\text{C}$ . inclusive at intervals of  $5^{\circ}$ . At each temperature, pots containing soil without cellulose and pots containing soil plus 1 per cent by weight of cellulose in the form of finely ground wheat straw were incubated for 7 weeks. The results of experiment 1 are shown in figure 1.

Throughout the experiment the nitrate content of the soil containing cellulose was definitely lower than that of the soil without cellulose. There was no recovery of the nitrate content in the soil containing cellulose at any time during the experiment. There is an apparent lack of uniformity in the results illustrated in figure 1. This may have been due to the necessity of drawing the graph on an exaggerated scale, because of the small amount of nitrates present, in order to show the relationships between soil with and soil without cellulose at the different temperatures.

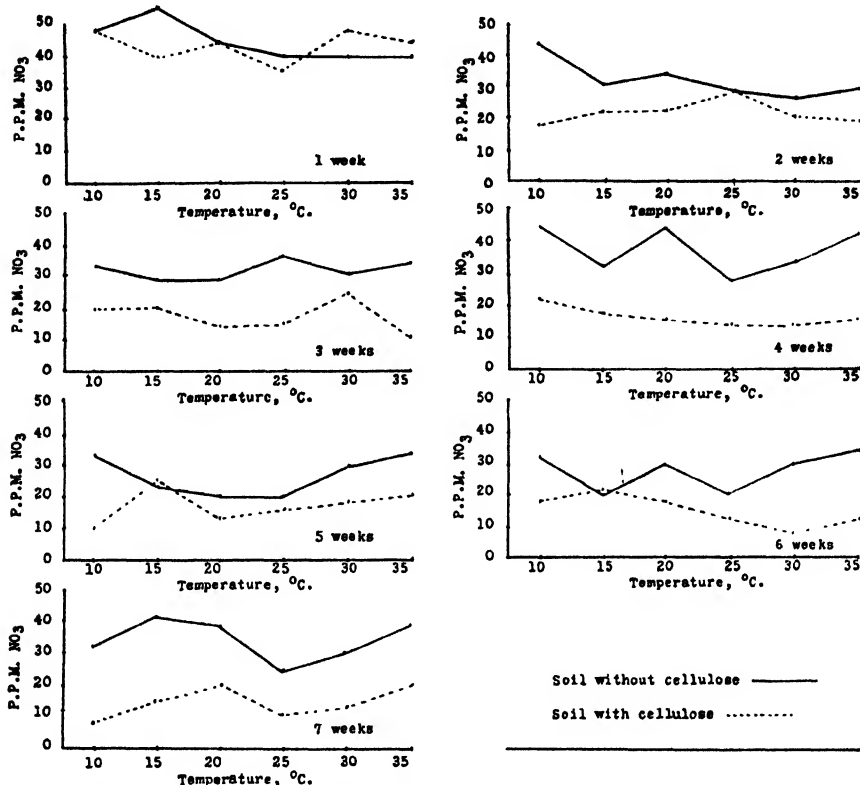


FIG. 1. NITRATE AT DIFFERENT TEMPERATURES IN A NITROGEN-POOR SOIL WITHOUT AND WITH ADDED CELLULOSE

The nitrate level of the soil in all of the pots was considerably lower than the initial nitrate content of the soil employed. This indicated some loss of nitrate in all of the pots, the loss in the presence of cellulose being greater than that in the absence of cellulose. The loss of nitrate could not have been due to leaching because the pots were not drained.

### Experiment 2

Since the soil employed in experiment 1 had never been limed it was decided to investigate the influence of calcium salts on the nitrate content of soil in

the presence and absence of cellulose. The nitrate content of the soil employed was 70 p.p.m. Pots were prepared according to the following plan: soil untreated, soil plus calcium carbonate, soil plus calcium sulfate, soil plus cellulose, soil plus cellulose and calcium carbonate, soil plus cellulose and calcium sulfate. The calcium salts were added at the rate of 5 gm. per kilogram of soil. One per cent of finely ground wheat straw was added for cellu-

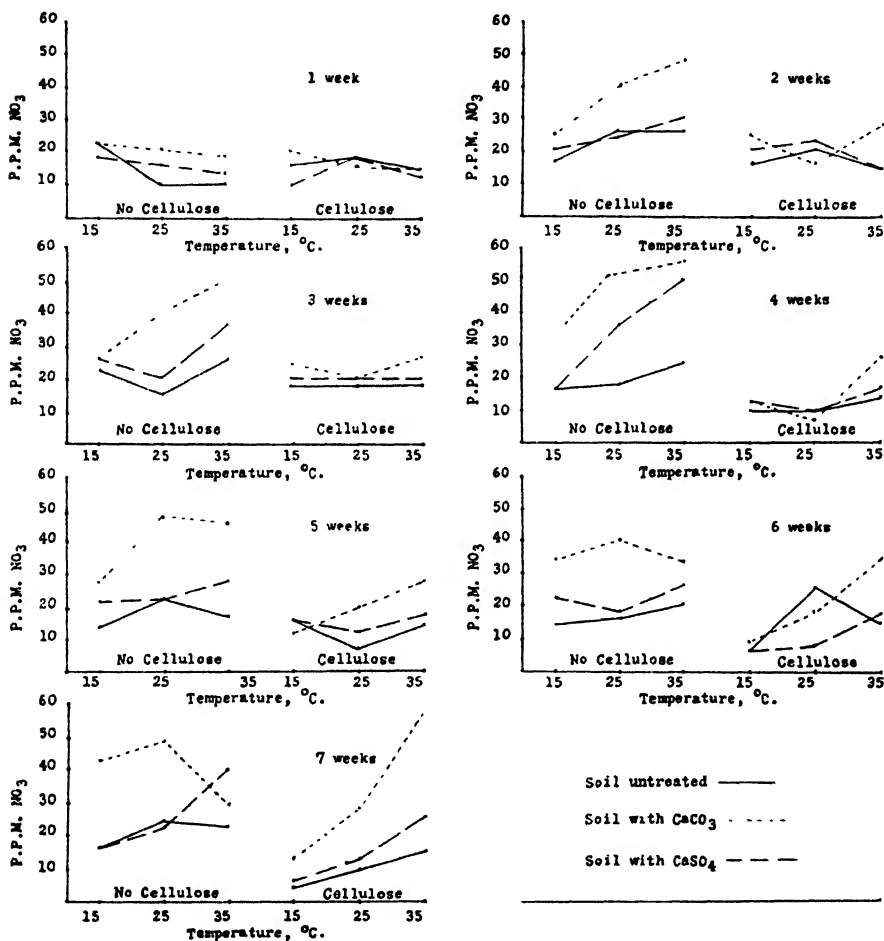


FIG. 2. NITRATE AT DIFFERENT TEMPERATURES IN A NITROGEN-POOR SOIL WITHOUT AND WITH ADDED CELLULOSE, AND TREATED WITH  $\text{CaCO}_3$  OR WITH  $\text{CaSO}_4$

lose. Pots representing each of the treatments were incubated at each of three temperatures, 15°, 25°, and 35°C. Limitation of equipment made it impossible to use the entire temperature range employed in the first experiment, so temperatures were chosen to represent the low, median, and high of the temperature range previously employed. The incubation period was 7 weeks as before. The results of experiment 2 are shown in figure 2.

In soil with and in soil without cellulose, neither of which contained added calcium, the observations made agreed with those from experiment 1. In the latter half of the experiment at 20°C. and above, there was less nitrate depletion in soil containing calcium carbonate plus cellulose than in soil containing cellulose and no calcium carbonate. The greatest concentration of nitrate was observed in soil at 35°C. At 15°C. there was some depletion of the soil nitrate in soil containing calcium carbonate and no cellulose. In the same soil, during the latter half of the experiment, and at 25° and 35°C., the nitrate content of soil remained nearly equal to the initial nitrate content of the soil. In general it may be stated that calcium carbonate was instrumental in maintaining a higher nitrate content in soil in both the presence and absence of cellulose, than was apparent in soil with parallel treatment except that added calcium carbonate was lacking. The nitrate content of soil containing calcium carbonate and cellulose remained lower than that of soil containing calcium carbonate and no cellulose. Calcium sulfate had no significant effect.

The influence of the added calcium salts on the pH values of the soil is shown in table 2.

TABLE 2  
*The pH value of soils of experiment 2 after 7 weeks' incubation*

TREATMENT OF SOIL	pH AT 15°C.	pH at 25°C.	pH at 35°C.
No treatment . . . . .	6 1	5 9	5 7
CaCO <sub>3</sub> . . . . .	6 6	6 4	6.2
CaSO <sub>4</sub> . . . . .	6 1	6 0	5 6
Cellulose . . . . .	6.1	6 0	6.0
Cellulose and CaCO <sub>3</sub> . . . . .	6 0	6 5	6.5
Cellulose and CaSO <sub>4</sub> . . . . .	5 7	6 0	5.6

### *Experiment 3*

Since the soil employed in experiment 1 was low in nitrogen and total nitrates, a well-seasoned compost soil was chosen for the third experiment in order that results obtained from soils of high and low nitrate and nitrogen content might be compared. The compost had been made by combining manure with soil of the same quality as that used in the first two experiments. No lime had been applied to the soil. The experiment was prepared in the same manner as experiment 1, except that finely cut paper toweling, instead of straw, was used for cellulose. Straw appeared to be better adapted than paper for use with poor soil because straw contained some nitrogen which could be converted into available nitrogen for the use of the cellulose decomposing organisms. The compost soil naturally had no need for additional nitrogen, so paper was substituted for the straw. The incubating temperatures were the same as those employed in the first experiment, 10° to 35°C. inclusive at intervals of 5°. The incubation period was 7 weeks. The results of the experiment are shown in figure 3.

In this experiment at temperatures below 25°C., the nitrate content of soil containing cellulose was definitely less than that of soil without cellulose. This agrees with the results obtained in the first experiment. During the latter half of the third experiment at 25°C. and above, the nitrate content of soil containing cellulose approached, and in some cases was equal to, the nitrate content of soil without cellulose. This observation indicated the ability of a soil rich in organic nitrogen to produce nitrate in sufficient quantity to replace that lost as a result of cellulose decomposition. It might be argued that the higher

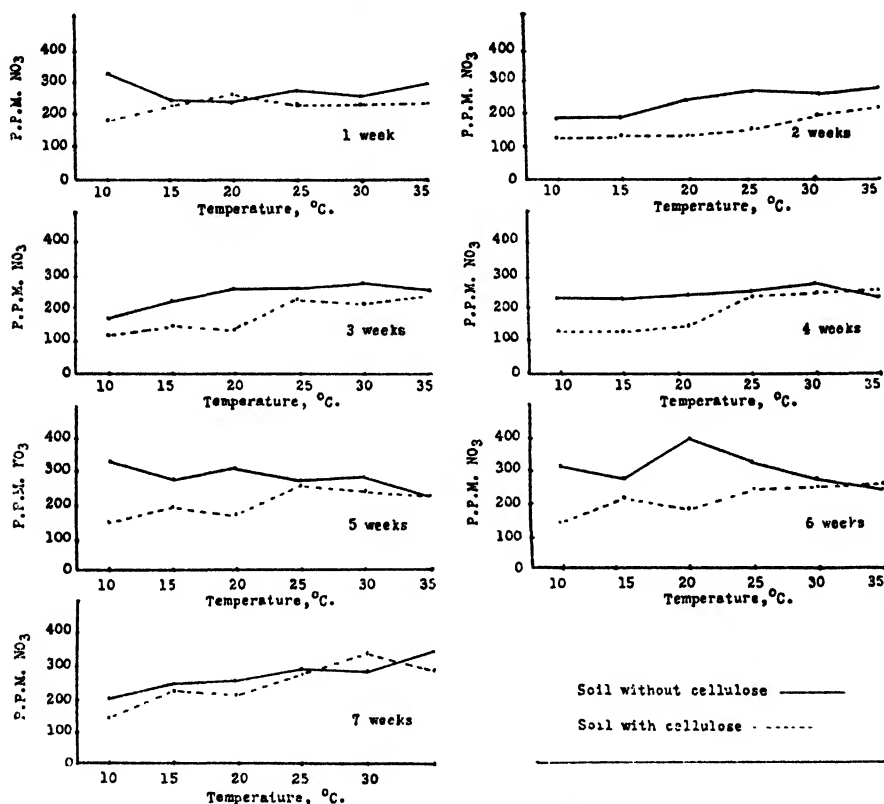


FIG. 3. NITRATE AT DIFFERENT TEMPERATURES IN A COMPOST SOIL WITHOUT AND WITH ADDED CELLULOSE

nitrate content observed under the conditions mentioned may have been due to a failure of the microorganisms to utilize the nitrate of the soil, rather than to increased nitrification. A comparison of the findings of experiment 1 with those of experiment 3 seem to indicate that active nitrate depletion had taken place and that the nitrate lost had been replaced. Certainly nitrate depletion was indicated in experiment 1.

In the absence of cellulose the greatest concentration of nitrate was observed at 20° to 30° inclusive. When cellulose was present the maximum amount of



nitrate was observed at 25° to 35°C. inclusive. During the latter half of the experiment at 25°C. and above, the nitrate content of soil with and of soil without cellulose exceeded the initial nitrate content of the soil (220 p.p.m.).

The most important observation in this experiment was the ability of soil rich in organic nitrogen to maintain a high nitrate content in the presence of decomposing cellulose, particularly at higher temperatures and during the latter half of the experiment.

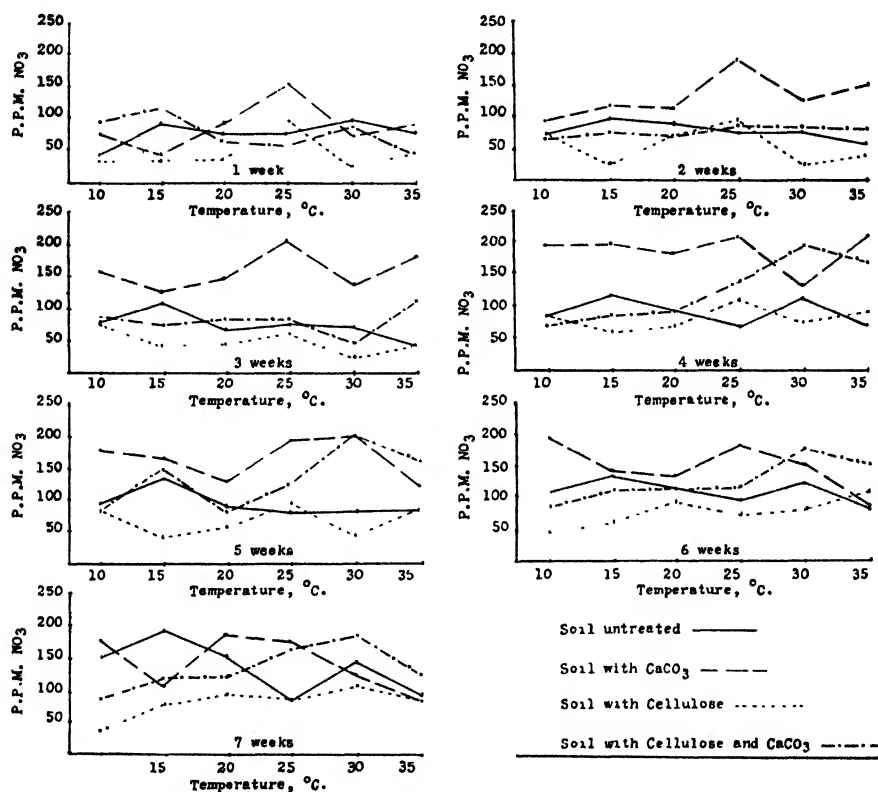


FIG. 4. NITRATE AT DIFFERENT TEMPERATURES IN A SOIL OF MEDIUM NITROGEN CONTENT, WITHOUT AND WITH ADDED CELLULOSE, AND TREATED WITH  $\text{CaCO}_3$

#### Experiment 4

In order to study the influence of added calcium salts under the conditions of experiment 3, an experiment was planned similar to experiment 2. Since the compost employed in the third experiment was not available, a soil was chosen containing 95 p.p.m. of nitrates and 1.5 mgm. of total nitrogen per gram of soil. Wheat straw was again employed for cellulose. It was thought that the nitrogen of the soil together with that of the straw should furnish sufficient nitrogen to indicate any ability the soil might have to produce nitrates under the conditions of the experiment. This proved to be true.

The experiment was set up as follows: untreated soil, soil plus calcium carbonate, soil plus cellulose, soil plus cellulose and calcium carbonate. Calcium carbonate was added at the rate of 5 gm. per kilogram of soil. Calcium sulfate was not employed because it had had little apparent influence when used in the second experiment, and by its elimination it was possible to employ the same temperature range as that used in the third experiment. One per cent by weight of cellulose was used. Pots representing each of the aforementioned treatments were incubated at each of six temperatures, 10° to 35°C. inclusive, at intervals of 5°. Incubation was continued for 7 weeks. The results of experiment 4 are shown in figure 4.

In pots without calcium carbonate the observations made in the third experiment were repeated but on a less extensive scale because of the lower initial nitrate and nitrogen content of the soil. The addition of calcium carbonate to soil without cellulose resulted in an increase of nitrate above the initial nitrate content of the soil at and above 20°C. The nitrate content of the soil was slightly less at 35° than at 20° to 30°C. inclusive. The addition of calcium carbonate to soil containing cellulose appeared to simulate the increase

TABLE 3  
*The pH value of soils of experiment 4 after 7 weeks of incubation*

TREATMENT OF SOIL	pH AT 10°C.	pH AT 15°C.	pH AT 20°C.	pH AT 25°C.	pH AT 30°C.	pH AT 35°C.
No treatment	6.2	5.8	5.9	5.8	6.0	6.1
CaCO <sub>3</sub>	6.9	6.7	6.9	6.8	6.9	6.9
Cellulose . .	5.8	6.4	5.7	5.8	5.8	6.0
Cellulose and CaCO <sub>3</sub>	6.8	7.0	6.8	6.7	6.8	6.9

of nitrate during the latter half of the experiment at 25°C. and above. The nitrate concentrations of soil containing calcium carbonate, both with and without cellulose, were practically equal at 25°C. and above, during the latter half of the experiment. These concentrations were considerably above the initial nitrate concentration of the soil. Apparently 35° was a less favorable temperature than 25° and 30°C. for a high nitrate concentration in the soils.

The principal observation from this experiment was that at 25°C. and above the addition of calcium carbonate to soil with or without added cellulose resulted in an increase in the amount of nitrate in the soil. The influence of calcium carbonate on the pH values of the soil is shown in table 3.

#### PART II. INFLUENCE OF ADDED CELLULOSE ON SOIL NITRATE AS INDICATED BY THE DRY WEIGHT OF TOMATO PLANTS

The experiments of part I with soils receiving applications of cellulose, calcium carbonate, and calcium sulfate were carried on in containers from which samples were taken each week for nitrate determinations. It was not possible to grow plants in these containers as the sampling procedure would have muti-

lated the root systems. Consequently a parallel series of soils containing plants constituted one-half of each experiment. This series constitutes part II of the study. The soil in the containers with the plants was not disturbed. It is assumed that the processes taking place in the fallow containers were duplicated very closely in the parallel series containing plants.

TABLE 4

*Relative dry-weight yields of tomato plants from soils of low and high initial nitrate content at different temperatures. With and without cellulose, and with and without calcium carbonate and calcium sulfate*

TEMPERATURE, °C.	INITIAL NO <sub>3</sub> CONTENT OF SOIL	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
		54 p.p.m.		70 p.p.m.		220 p.p.m.		95 p.p.m.	
		Relative values (100 = 11.0 gm.)*		Relative values (100 = 9.8 gm.)		Relative values (100 = 54.6 gm.)		Relative values (100 = 5.7 gm.)	
		Without cellulose	With cellulose	Without cellulose	With cellulose	Without cellulose	With cellulose	Without cellulose	With cellulose
10	None	17	15			3	3	23	9
	CaCO <sub>3</sub>							19	9
15	None	32	17	28	27	71	56	26	28
	CaCO <sub>3</sub>			54	16			37	39
	CaSO <sub>4</sub>			27	15				
20	None	33	11			85	90	49	49
	CaCO <sub>3</sub>							95	67
25	None	53	15	73	43	103	90	86	124
	CaCO <sub>3</sub>			164	77			118	144
	CaSO <sub>4</sub>			80	28				
30	None	81	35			109	109	118	142
	CaCO <sub>3</sub>							141	218
35	None	100	40	100	97	100	113	100	148
	CaCO <sub>3</sub>			186	169			137	167
	CaSO <sub>4</sub>			85	38				

\*The absolute weight in grams of the plant may be obtained by multiplying the weight at the head of the tabulation of each experiment by the relative value divided by 100.

The tomato plant was used as an indicator plant. Three plants were set in each container. Because of the upright growth habit of the tomato plant there was little danger of one set of plants shading an adjoining set. The experiments were set up at the same time as those of part I. They were continued for 7 weeks and concluded on the day of the seventh sampling of the soils from the pots of part I. At the conclusion of each experiment, the plants were measured, cut at the surface of the soil, dried at 101°C., and weighed. Table 4

presents a composite tabulation of the weights from the four experiments. These weights are in terms of relative values with the weight of the plants in a non-cellulose soil at 35°C. equal to 100. In three of the four experiments, this temperature gave the highest yield in the absence of any soil treatment.

Cellulose mixed with a soil of low nitrate content considerably reduced the dry weight of the plants. This was particularly true at the temperature optimum for the growth of the tomato plant, which is close to 25°C., according to Jones, Johnson, and Dickson (8), and to Godfrey (5). However, if the soil had a very high level of fertility, as was the case in experiment 3, cellulose had practically no effect on the dry weight of the plants. The dry weight increased directly as the soil temperature was raised, and this increase in weight, in the soil without added cellulose, was uniformly paralleled in the series to which cellulose had been added. The nitrate content of this soil was markedly reduced by the added cellulose, but the low nitrate levels attained were still above the requirements of the plants in every case. The effect of cellulose on the yield of dry matter in experiment 4 presents a situation that must be interpreted on the basis of temperature effect on the plant and microbial activity in the soil. The initial nitrate content of this soil was neither high nor low and the crop employed was sensitive to the variations of the nitrate content as affected by temperature and the added cellulose and calcium carbonate. It seems quite apparent that nitrification was active at temperatures of 25°, 30°, 35°C., and this nitrification was more intense in the soils to which cellulose had been added. At temperatures of 15° and 20°C., there was no effect due to added cellulose, but at the lowest temperature, 10°C., the cellulose materially reduced the nitrate content of the soil, which was reflected in a lower crop yield.

The effect of calcium carbonate on dry weight of the plants was more apparent at the higher temperatures than at the lower temperatures. In a soil with an initial low nitrate content (experiment 2) calcium carbonate stimulated nitrification in both the presence and absence of added cellulose, but the increase of nitrates was greater in the soils to which no cellulose had been added. In soil of fair nitrate content (experiment 4) calcium carbonate was ineffective at the lower temperatures of 10° and 15°C.; but at temperatures of 20°C. and above, the dry weights of the plants indicated increased nitrification. In experiment 4 the nitrification was intensified by the presence of added cellulose. This was not the case in experiment 2 which employed a soil with a very low nitrogen content.

Calcium sulfate, added to the soil in an amount sufficient to give a calcium content equal to that in calcium carbonate, seemed to have a depressing effect on the yield of dry plant material, but this depressing effect is not correlated with any effect of calcium sulfate on the nitrate content of the soil. The calcium sulfate was used in experiment 2, and, as it did not prove to be a substitute for calcium carbonate, it was not used in the succeeding experiments. Calcium sulfate had a depressing effect in the soil to which cellulose had been added.

## DISCUSSION

The results in the first experiment showing that there was less nitrate in soil containing cellulose than in soil without were to be expected and are in agreement with the experimental findings of other investigators and with agricultural practise. If the experimental results reported in this paper are to be significant, they should demonstrate conditions under which the nitrate content of soil can be maintained or increased in the presence of cellulose. The second, third, and fourth experiments appear to demonstrate such conditions.

In the second experiment there was less loss of nitrate, during the latter half of the experiment at 25°C. and above, from soil containing cellulose and calcium carbonate than from soil containing cellulose and no calcium carbonate. Under these conditions 35°C. appeared to be a more favorable temperature than 25°C. for increased nitrate. In the third experiment in which cellulose was added to soil rich in organic nitrogen there was sufficient nitrification at 25°C. and above to replace the nitrate lost as a result of cellulose decomposition. This was particularly true during the latter half of the experiment. In the fourth experiment the addition of calcium carbonate to soil with and to soil without cellulose resulted in a nitrate concentration in the soil under either condition which was greater than the initial nitrate concentration of the soil employed, and which was in contrast with the loss of nitrate in the same soil containing cellulose but no calcium carbonate.

The observation in the second and fourth experiments that the presence of calcium carbonate in soil results in increased nitrate content agrees with the statement of Noyes and Conner (9) who reported that calcium carbonate caused an increase in nitrification in soil.

Panganiban (11) and Russell, Jones and Bahrt (12) found the optimum temperature for nitrification to be 35°C. In the experiments reported in this paper 25° and 30°C. proved in most instances to be the more favorable temperatures for increase in nitrate.

Jenny (6) stated that it is easier to build up a soil in a warm climate than in a cooler one. No doubt this is true in general, but the first two experiments here reported indicate that it would not be advisable to plow straw into a poor soil regardless of the temperature. Not even the addition of lime would prevent serious nitrate depletion in such a soil.<sup>4</sup>

<sup>4</sup> Visual evidence was depended upon in determining the fact that the cellulose was decomposed in the soils during the course of the experiments. At the beginning of each experiment the cellulose could be seen plainly in any sample of the soil. At the end of each experiment practically no cellulose was visible in soil held at the higher temperatures, and very little in soil held at 15°C. and below. This was equally true of straw and paper.

In order to investigate the influence of temperature on the rate of cellulose decomposition the following experiment was planned: Culture tubes were prepared containing medium no. 85 from the Fred and Waksman (4) laboratory manual. Strips of filter paper were inserted into the tubes so that a part of the paper in each tube was immersed in the fluid medium.

## SUMMARY

The influence of temperature on the nitrate content of soil in the presence of decomposing cellulose has been investigated. Soils low and soils high in organic nitrogen and with and without added cellulose were maintained at temperatures of from 10° to 35°C. for a period of 7 weeks. A series of fallow soils was paralleled by a series of soils from the same sources which were identically treated and which contained tomato plants. Certain of the soils of these two series were treated with calcium carbonate or with calcium sulfate.

In soil which was low in organic nitrogen the addition of cellulose resulted in a depletion of nitrate at all temperatures. Calcium carbonate added to this soil in the presence of added cellulose reduced the nitrate depletion during the latter half of the time period at 20°C. and above. Calcium sulfate did not prove to be a substitute for calcium carbonate.

In soil which was rich in organic nitrogen, added cellulose resulted in nitrate depletion at all temperatures during the first half of the time period. During the latter half of the time period at temperatures of 25°C. and above the nitrate content of the soil containing added cellulose showed a definite tendency to equal the nitrate content of the same soil without added cellulose. The addition of calcium carbonate to this soil with and without added cellulose stimulated nitrification.

The crop response as indicated by the dry weights of the tomato plants was in close agreement with what would be expected from the nitrate determinations made from the fallow series. The dry weight of the plants was considerably reduced by the addition of cellulose to a soil low in nitrogen. In a soil high in organic nitrogen the addition of cellulose reduced the nitrate content, but not to a level that impoverished the soil for the requirements of the crop. Calcium carbonate stimulated greater growth of the plants at the higher temperatures in both the presence and absence of added cellulose. Calcium sulfate seemed to have a depressing effect on the yield of dry plant material.

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The tubes were sterilized and inoculated with 1 gm. each of soil known to possess active cellulose-decomposing properties. The tubes were buried in the soil of the various pots to provide incubation at the different temperatures employed in the experiments reported. The tubes were bent so that most of the length was horizontal at a depth of 2 inches below the surface of the soil, with only the plugged end of each tube protruding. After 10 days' incubation the tubes were examined. Cellulose decomposition was indicated by decomposition of the paper at the surface of the fluid in the tubes. The cellulose-decomposing activity appeared to be equally vigorous at all temperatures except at 7° and 10°C., where decomposition was evident but retarded.

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## PLATE 1

### CONSTANT SOIL TEMPERATURE APPARATUS

FIG. 1. Complete apparatus.

FIG. 2. A unit of the apparatus. The bimetallic thermo-regulator, 1, controls the relay, 2, which cuts in or out the current to the strip heaters, 3. The strip heaters are inserted in the oven, 4, which is a cell extending into the tank, 10. The thermometer, 5, indicates the temperature of the water bath. Cooling water is obtained from the main at 7 and enters a pipe at 6 which leads to the bottom of the tank where it creates circulation currents and overflows at 8. The soil containers number four in each unit and may be easily removed as is shown at 9.



FIG. 1

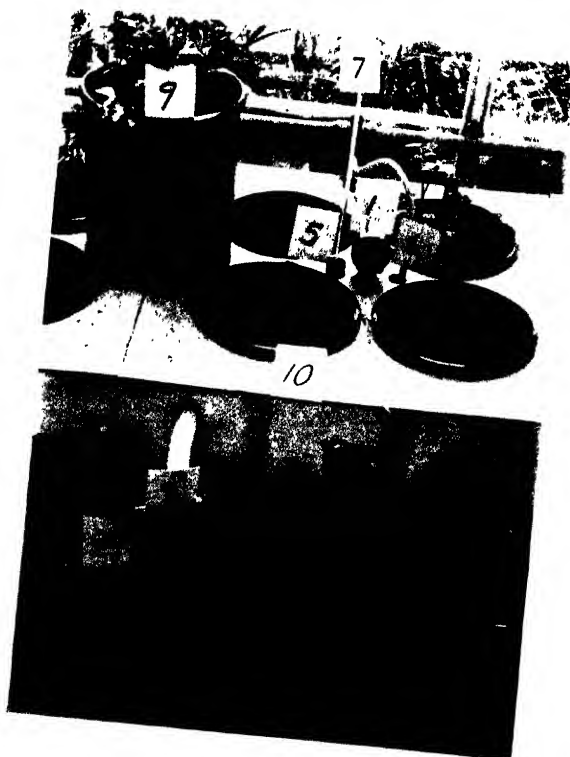


FIG. 2





# CAUSES OF LOW NITRIFICATION CAPACITY OF CERTAIN SOILS<sup>1</sup>

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In connection with some studies of nitrification in Texas soils, it was found that a number of soils had no power to nitrify ammonium sulfate. Small amounts of nitrates would be produced from the organic matter of the soil, but where ammonium sulfate was added, the quantity of nitrates would frequently be less than when no addition was made. Stevens and Withers (8) reported that a number of North Carolina soils failed to nitrify organic nitrogen, but offered no explanation. Lipman (6), Sackett (7), Kelly (5), and perhaps others reported soils which did not nitrify. Gainey (4) claimed that the failure to obtain nitrification was due to the particular experimental conditions and not to the entire absence of active organisms. In the course of our work a number of soils were found which, although they usually produced some nitrates from the soil nitrogen, did not nitrify ammonium sulfate. Because it seemed a matter of some importance to find out why these soils failed to nitrify, extensive experiments were conducted for this purpose.

## METHOD OF WORK

The method used was similar to that usually followed (10): 200 gm. of soil was mixed with inoculating liquid, 10 cc. of ammonium sulfate solution containing 0.1 gm. nitrogen, water equal to 50 per cent of the water capacity of the soil, placed in a 300-cc. tall Pyrex beaker, weighed and incubated at 35°C. Another portion received inoculating liquid and water only. The water lost by evaporation was replaced twice a week. At the end of 28 days, nitrates and nitrites were estimated by the methods already described (3). This general method was modified according to the conditions being studied.

## EFFECT OF BACTERIA AND CALCIUM CARBONATE

After a considerable amount of preliminary work, which will not be discussed, we found that, by additions of portions of actively nitrifying cultures, of calcium carbonate, or of both nitrifying cultures and calcium carbonate, ammonium sulfate could be made to nitrify in soils which otherwise did not oxidize it. A large number of soils were found which did not nitrify ammonium sulfate under the usual conditions, but more than 90 per cent of these soils

<sup>1</sup> Technical Contribution No. 207, Texas Agricultural Experiment Station, presented at the New Orleans meeting of the American Chemical Society, March 30, 1932, and revised.

had a high nitrifying power when inoculated with nitrifying organisms, or when they received calcium carbonate, or both.

Table 1 contains some of the results which show this to be the case. The first soil, Susquehanna fine sandy loam, 0-7 inches, did not nitrify ammonium sulfate alone or with additions of calcium carbonate or of nitrifying soil. When both calcium carbonate and nitrifying soil were added, it nitrified a high percentage of the nitrogen in the ammonium sulfate.

TABLE 1

*Effect on nitric nitrogen produced of addition of cultures and calcium carbonate to soils low in nitrifying power*

ADDITION TO 200 GM. SOIL	NITRIC NITROGEN (P P M) PRODUCED IN									
	35172 Susquehanna fine sandy loam, 0-7"	35170 Crockett fine sandy loam, 0-7"	31889 Webb fine sandy loam, 7-19"	32645 Lake Charles clay loam, 7-19"	32648 Lake Charles very fine sandy loam, 13-22"	33128 Garner clay, 0-7"	33130 Bowie fine sandy loam, 3-7"	33135 Susquehanna fine sandy loam, 0-7"	33137 Wilson clay, 0-7"	33712 Houston black clay, 18-24"
None . . . . .	18	37	20	7	0	0	0	60	22	23
Ammonium sulfate (0.1 gm. N). . . . .	14	49	30	21	2	0	0	92	19	41
Calcium carbonate (2 gm.).	31	49	44	1	0	10	55	110	44	...
Calcium carbonate and ammonium sulfate . . .	37	400	47	1	0	33	61	172	53	..
Nitrifying soil (10 gm.).	42	..	86	..	34	115	65	92	40	68
Nitrifying soil and ammon- ium sulfate. . . . .	70	..	550	156	294	430	410	128	53	340
Nitrifying soil and calcium carbonate . . . . .	44	.	.	512	40	160	.	108	120	...
Nitrifying soil, calcium carbonate and ammon- ium sulfate. . . . .	320	...	...	..	370	600	...	525	360	...
Nitrifying soil sterilized. .	...	...	58	0	...	28	..	525	20	54
Nitrifying soil sterilized and ammonium sulfate .	...	...	58	0	...	30	...	215	32	62

The second soil, Crockett fine sandy loam, 0-7 inches, did not nitrify alone but nitrified when calcium carbonate was added. Although it is well known that calcium carbonate would accelerate nitrification (10), it has not been recognized that calcium carbonate would cause soils to nitrify which otherwise did not nitrify ammonium sulfate at all.

The third soil, Webb fine sandy loam, subsoil 7-19 inches, had little nitrifying power alone or with calcium carbonate added, but when nitrifying soil was added, it developed a high nitrifying power. Results with other soils representing the various groups are given in table 1. Nitrification in all these soils

originally was low, but was decidedly increased by the nitrifying cultures or the calcium carbonate.

Of the large number of soils studied, 53 are classified, as shown in table 2. One-third of the 21 surface soils listed have a high nitrification power, while one-third have no nitrification power for ammonium sulfate. Approximately half of these are made to nitrify well by additions of calcium carbonate and one-half by additions of calcium carbonate together with soil containing actively nitrifying bacteria. Of the 32 subsoils, about 16 per cent have a high nitrifying power while 60 per cent have no nitrifying power for ammonium sulfate. Of those which have little or no nitrifying power, two, or about 10 per cent, are made to have high nitrification by bacteria alone, 1, or 5 per cent, by calcium carbonate, and 16, or 85 per cent, require both bacteria and calcium carbonate. The subsoils are much more frequently deficient in bacteria than

TABLE 2

*Number of samples whose nitrification was increased by lime or bacteria compared to total number*

	NUMBER OF SURFACE SOILS	NUMBER OF SUBSOILS
Nitrification capacity 70-100 . . . . .	7	5
Nitrification capacity 50-70 . . . . .	2	4
Nitrification capacity zero . . . . .	(7)	(19)
Made high by bacteria alone . . . . .	0	2
Made high by lime alone . . . . .	4	1
Made high by lime and bacteria . . . . .	3	16
Nitrification 1-20 . . . . .	(5)	(4)
Made high by bacteria alone . . . . .	1	2
Made high by lime alone . . . . .	2	2
Made high by lime and bacteria . . . . .	2	0
Total number . . . . .	21	32

the surface soils, but the number of surface soils deficient in nitrifying bacteria is comparatively large.

The nitrifying soil, of course, in addition to the nitrifying organisms, may carry soil materials which affect nitrification. The nitrifying organisms are usually the chief addition, as is shown when a portion of the nitrifying soil is sterilized, and added in the same way as the unsterilized culture. A few of such results are given in table 1. It is seen that the sterilized soil did not produce nitrification. Other experiments could be cited to the same effect. Of course, if the sterilized soil contained calcium carbonate in sufficient quantity, it might cause nitrification to take place in soils which need only calcium carbonate for this purpose, or which need calcium carbonate in addition to the bacteria.

The nitrifying soils used were portions of cultures obtained in previous nitrification experiments conducted by the methods previously described, and

contained some nitrates which of course can be allowed for by blank tests. If the soil needed calcium carbonate for nitrification, appreciable amounts of calcium carbonate in the nitrifying soil would affect the nitrification. In case it is desired to know whether the addition of nitrifying organisms produces nitrification without additions of calcium carbonate also, it is necessary to select cultures low in calcium carbonate for the inoculation. The effect of the other ingredients of the inoculating soil is being studied.

#### EFFECT OF INOCULATING LIQUID

It is customary to inoculate the soils used in nitrification experiments with liquids prepared from nitrifying soils (10). Inoculating liquid was likewise

TABLE 3  
*Effect of inoculating liquid upon production of nitrates, in parts per million of soil*

LABORATORY NUMBER	SOIL TYPE, LOCATION AND DEPTH	NITRATES PRODUCED WITH AMMONIUM SULFATE		GAIN DUE TO INOCULATING LIQUID	NITRATES PRODUCED WITHOUT AMMONIUM SULFATE		GAIN DUE TO INOCULATING LIQUID
		Plus inoculating liquid	No inoculating liquid		Plus inoculating liquid	No inoculating liquid	
6731	Surface soil, Willacy Co., 0-6"	210	260	0	105	96	9
7355	Surface soil, Runnels Co., 0-6"	460	420	40	220	220	0
8815	Surface soil, Coryell Co., 0-4"	537	550	0	160	136	24
12533	Wilson loam, Ellis Co., 0-6"	600	600	0	152	160	0
12534	Wilson loam, Ellis Co., 6-18"	450	460	0	94	96	0
12576	Durant fine sandy loam, Ellis Co., 0-12"	400	390	10	122	112	10
29423	Upland blackland, Bell Co., 0-12"	331	275	56	47	51	0
29424	Bottomland, Bell Co., 0-12"	550	550	0	100	102	0
31884	Hidalgo clay loam, Frio Co., 0-7"	600	575	25	107	107	0
33702	Wilson clay, Collins Co., 0-7"	215	210	5	84	80	4
33709	Trinity clay, Collins Co., 7-24"	550	550	0	105	105	0
33710	Houston black clay, Collins Co., 0-7"	450	470	0	60	61	0

used in the work here reported and the discovery that the low nitrification in many soils was due to deficiency in nitrification bacteria was delayed because it was at first thought the organisms were being added in sufficient quantity by this inoculating liquid. After the great effect of additions of bacteria in cultures of nitrifying soils was discovered, it seemed desirable to ascertain the effect of the addition of inoculating liquid. The inoculating liquid was prepared by mixing 100 gm. of fresh field soil with 200 cc. of water; 10 cc. of the supernatant liquid was added to each culture when it was used. Air-dried soils which had been stored in the laboratory were used, with the results given in table 3.

It is apparent that the inoculating liquid had little or no effect upon the nitrification in these soils, and that the nitrification which occurred was brought

about by bacteria already present in the air-dried soil. If the inoculating liquid were thoroughly shaken and drawn off with the soil in suspension, it would no doubt carry some nitrifying organisms, but this amount would probably vary with the quantity of soil in suspension, and might be different for different portions of the same inoculating liquid. It appears that this method of adding nitrifying bacteria to the soil is not satisfactory.<sup>2</sup>

#### PERSISTENCE OF NITRIFYING BACTERIA IN DRY SOIL

The fact that the nitrification was caused by the bacteria in the dry soil is interesting in view of the fact some of these soils had been stored for long periods of time. Soil 6731 had been stored for 18 years; soil 7355, for 17 years; soil 8815, for 16 years; and soils 12533, 12534, and 12576, for 14 years. Although the samples were kept under laboratory, and not aseptic, conditions, and may have received small amounts of bacteria from the air during the period, the amount so received would hardly be sufficient to account for the quantities of nitrifying organisms present. Most of them must have persisted in the soil.

#### EFFECT OF NUMBER OF BACTERIA ADDED

It appeared from the results just presented that the results of a nitrification experiment might depend to a considerable extent upon the number of nitrifying bacteria present in the soil at the beginning of the experiment. It also seemed possible that different kinds of soil might give different results with the same number of added bacteria. Soils of low nitrifying power might require larger numbers of bacteria to produce the same amount of nitrates than soils with high nitrifying power.

For this reason, experiments were made by using different quantities of the same culture to supply different numbers of soil bacteria. In one series, different quantities of cultures from different soils were added to the same sterilized soil; in another series, the same culture was inoculated into different sterilized soils. Sterilization was effected by heating 2 hours at 140°C. in an electric oven.

Results on soils representative of the two series are shown in figures 1 and 2. The quantities of nitrates usually increased rapidly with the small additions of soil, but when more than 1 gm. of inoculant was added to 200 gm. of soil, the increase caused by the subsequent addition was low compared with the number of organisms added. The action of the bacteria followed the law of diminishing returns, in that each successive increment produced less increase in nitrification than the preceding addition. The nitrification was rarely in direct proportion to the quantity of inoculant added when it was small, that is, below 1 gm. to 200 gm. of soil, and never in proportion to the quantity added when the addition exceeded 1 gm. Hence it would not be correct to assume that the

<sup>2</sup> Since the foregoing was written, we have found that a suspension of 20 gm. of nitrifying soil in 500 cc. of water, added to a sterilized soil in quantities of 20 cc. or less, produces much greater amounts of nitrates than the corresponding quantity of the soil.

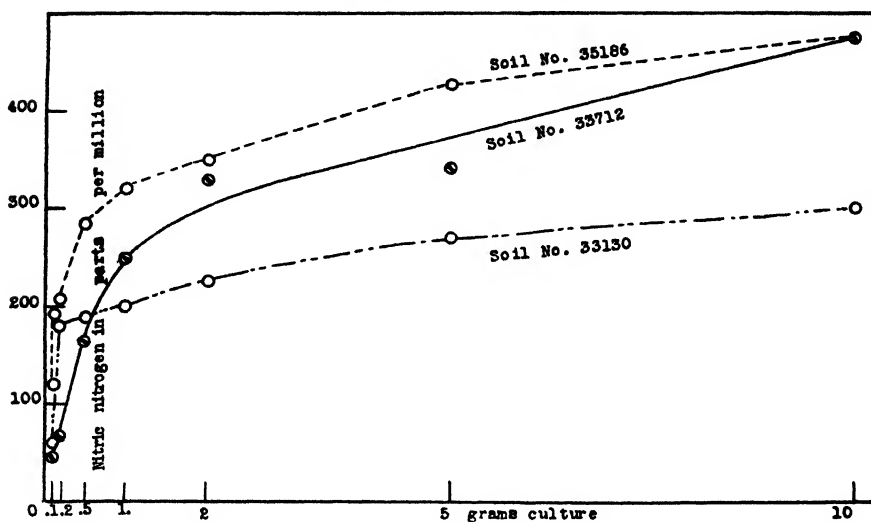


FIG. 1. RELATION OF THE NITRIC NITROGEN PRODUCED IN DIFFERENT STERILIZED SOILS TO THE QUANTITY OF THE SAME INOCULANT

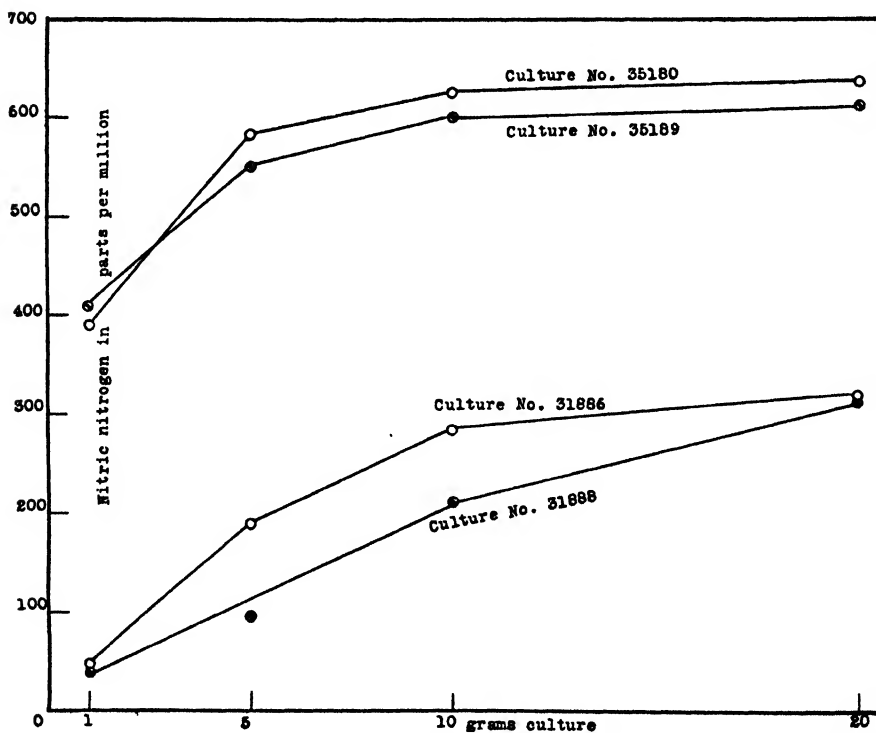


FIG. 2. RELATION OF THE NITRIC NITROGEN PRODUCED TO THE QUANTITY OF NITRIFYING CULTURES FROM DIFFERENT SOILS ADDED TO THE SAME STERILIZED SOIL

nitrates produced in an inoculated soil are in proportion to the number of bacteria present, or to use the quantity of nitrates as direct measure of the number of nitrifying bacteria in the inoculant.

In figure 1, the curves for the three soils are in two groups, parallel but widely separated, though the soils were sterilized and inoculated with the same culture. That is to say, the same quantities of nitrifying bacteria added to different soils produced different quantities of nitrates. This can, of course, be ascribed to differences in the chemical composition or physical properties of the two soils, and requires further study.

In figure 2, the same sterilized soil was inoculated with cultures from different soils. The four curves are in two groups similar in shape but they are located at different points on the scale. Cultures of soils 31886 and 31888 produce a maximum of 320 p.p.m. of nitric nitrogen, whereas cultures of soils 35180 and 35189 produce about 610. If the differences between culture 31886 and culture 35180 were due merely to a difference in the number of bacteria, we would expect these differences to be eliminated by the additions of increased quantities of culture 31886, and the same maximum to be attained. But increased additions of culture of soil 31886 had little effect, even though the quantity of nitrates produced was decidedly below the maximum produced by culture of soil 35180. Hence the differences must be due to differences in the nature of the bacterial complex in the two cultures, and not to differences in bacterial numbers. There must be decided differences in the bacterial complex in the two soils to produce such wide differences in the amounts of the end product. This difference might be due to different strains of nitrate and nitrite bacteria in the two cultures or to the presence of bacteria in culture 31886 which consume nitrates or nitrites as fast as they are formed after the high level is reached. It is a striking fact, as shown in figures 1 and 2, that similar results may be obtained with different cultures inoculated into the same sterilized soil, or by the same culture inoculated into different sterilized soils. In one case it is due to the bacterial complex, in the other case it is the character of the soil, which may act by modifying the bacterial complex.

The few other soils of high nitrification capacity which were tested responded to different amounts of inoculating culture in a similar way (fig. 1) to soil 35186. Soils of low nitrifying capacity, 33130 and 33712, at first produced less nitrate with the same number of organisms, but soil 33712 with 20 gm. inoculant produced as much nitrate as the soils of high nitrifying power, whereas soil 33130 did not. Other data regarding these two soils are given in table 1. Apparently, the same number of organisms may produce less nitrate in soils of low nitrifying capacity than in soils of high power, even though the difference may be small in some cases. This requires further study.

#### RELATION TO NITRITES

The foregoing discussion has related chiefly to nitrates. Nitrites, however, frequently occur in appreciable amounts in nitrification experiments. Since



we discovered this (1, 2), qualitative tests for nitrites have been made in all nitrification experiments, and quantitative estimations by methods already described (3), whenever appreciable amounts were found.

A number of soils which did not produce nitrates from ammonium sulfate alone or with calcium carbonate, produced large amounts of nitrites when calcium carbonate was added, showing that the nitrite organisms were present in sufficient number, even though the number of nitrate organisms was very low. Some of these soils are listed in table 4.

The sterilized soil inoculated with different amounts of cultures, as described in the preceding section, in many cases produced nitrites as well as nitrates. Some of these results are given in table 5. Generally the production of nitrites is highest with 0.1 gm. of inoculant to 200 gm. of sterilized soil, and decreases

TABLE 4  
*Production of nitrites induced by calcium carbonate*

LABORATORY NUMBER	SOIL	NITROUS NITROGEN		NITRIC NITROGEN	
		Alone	With ammonium sulfate	Alone	With ammonium sulfate
		p p m.	p p m.	p p m.	p p m.
33130	Bowie fine sandy loam, 3"-7"	0	168	55	61
33135	Susquehanna fine sandy loam, 7"-18"	0	106	19	24
35112	Miles fine sand, 0-7"	0	225	33	39
35113	Miles fine sand, 7"-24"	0	235	33	28
35114	Miles fine sand, 24"-36"	0	188	26	48
35168	Norfolk fine sand, 7"-36"	0	172	31	9
35169	Norfolk fine sand, 0-7"	0	245	38	33
35172	Susquehanna fine sandy loam, 0-7"	31	172	31	37
35184	Houston black clay, 24"-40"	4	69	9	17
35185	Tomlinson fine sandy loam, 21"-36"	0	288	17	17
35187	Tomlinson fine sandy loam, 7"-21"	0	152	21	40

with the increase in the amount of the inoculant, until practically no nitrite is produced with 2.0 gm. of inoculant. This would seem to indicate that either the nitrite organisms are more abundant in the inoculant than the nitrate organisms, or that they multiply more rapidly. Thus the low quantity of inoculant introduces sufficient numbers of nitrite organisms to produce appreciable amounts of nitrites, but not enough nitrate organisms to transform the nitrites into nitrates. Larger quantities of inoculant introduce more nitrate organisms, while the larger numbers of nitrite organisms so introduced are less effective. With 1 or 2 gm. of inoculant the nitrate organisms can transform all the nitrites produced into nitrates. This work also seems to indicate that the presence of high amounts of nitrites may be unfavorable to the growth of the nitrate organisms.

If we accept the general opinion that nitrogen must pass through the nitrous

stage before it becomes nitrates, the measure of nitrite production is the sum of the nitric and nitrous nitrogen. The production of nitrous nitrogen, measured in this way, is not in direct proportion to the number of organisms added in the inoculant. The first addition of 0.1 gm. of inoculant to 200 gm. of soil produces a high quantity of nitrites plus nitrates, whereas subsequent increments produce much smaller quantities. The effects of increments of inoculant are much less when nitrates and nitrites together are considered than when nitrates alone are used.

TABLE 5  
*Effect of amount of soil inoculant on nitrite formation (nitrogen parts per million)*

LABORATORY NUMBER	KIND OF NITROGEN PRODUCED	QUANTITY OF INOCULANT USED							
		None	0.1 gm.	0.2 gm.	0.5 gm.	1.0 gm.	2.0 gm.	5.0 gm.	10.0 gm.
33128	Nitric N	0	21	24	84	117	220	287	293
	Nitrous N	0	105	51	25	40	0	0	0
33330	Nitric N	6	56	180	190	200	225	270	300
	Nitrous N	11	140	46	54	57	41	4	0
33711	Nitric N	8	218	243	225	360	400	440	487
	Nitrous N	0	74	60	106	0	0	0	0
33712	Nitric N	3	53	64	176	250	330	340	475
	Nitrous N	5	225	184	82	3	0	0	0
35179	Nitric N	14	26	76	120	240	337	390	430
	Nitrous N	112	180	164	160	80	11	0	0
35180	Nitric N	8	105	112	285	360	430	500	512
	Nitrous N	28	148	156	28	0	0	0	0
35186	Nitric N	8	110	205	290	320	350	425	475
	Nitrous N	2	84	10	13	7	2	0	0
35188	Nitric N	2	43	72	136	250	320	390	437
	Nitrous N	7	200	188	168	66	12	0	0

#### RELATION TO NITRIFICATION EXPERIMENTS

From the foregoing discussion, it is apparent that when two field soils are compared in nitrifying power, the results chiefly measure the number of organisms in the soil at the time the work was begun, though not accurately. The need for calcium carbonate may decrease the accuracy of this measure in some soils. The comparison does not measure the initial reaction, buffer content, or presence of neutralizing substances in the soil (9), for although these may have something to do with the number of organisms in the soil at a particular time, other factors also affect the number of organisms, such as

temperature, aeration, and materials susceptible to nitrification. The number of organisms in two soils must be equalized before their capacity to nitrify can be compared.

It is important, in nitrification experiments, to know whether the difference in nitrification between two soils is due to differences in initial number of bacteria, to need or abundance of calcium carbonate, to a combination of both, or to other factors. It may then be necessary to attempt to equalize the differences. In certain types of experiments, soils of high nitrifying power, as determined by previous work, should be selected.

#### SUMMARY

A large number of samples of soils did not nitrify ammonium sulfate though they usually produced some nitrates from the soil nitrogen. Soils which do not nitrify ammonium sulfate may be caused to nitrify it by addition of cultures of actively nitrifying soil, of calcium carbonate, or of both nitrifying culture and calcium carbonate.

One-third of 21 surface soils have a high nitrifying power and one-third have practically no nitrifying power for ammonium sulfate by the method used. One-sixth of 32 subsoils had a high nitrifying power and 60 per cent had no nitrifying power for ammonium sulfate.

Approximately one-half of the 7 surface soils which did not nitrify ammonium sulfate could be made to have a high nitrifying power by additions of calcium carbonate, and the other half by additions of both calcium carbonate and soil cultures containing actively nitrifying bacteria. Of the 19 subsoils which did not nitrify ammonium sulfate, 2 assumed a high nitrifying power by additions of bacteria alone, 1 by addition of carbonate, and 16 by addition of both calcium carbonate and bacteria in a nitrifying culture.

Additions of sterilized cultures low in calcium carbonate did not bring about nitrification. The chief effect of the addition was due to the bacteria and not to other constituents of the added soil. The cultures were portions of soils receiving ammonium sulfate and nitrifying it to a high extent in a previous experiment.

Additions of inoculating liquid produced little nitrification. This is not a satisfactory method of adding nitrifying organisms to soils.

Nitrifying organisms may remain in a dry soil for many years.

The quantity of nitrates produced increased in general as the quantity of nitrifying cultures added to the soil increased from 0.1 to 20 gm. added to 200 gm. sterilized soil, but was not in direct proportion to the number of bacteria added. The quantity of nitrates is not suitable for use as a direct measure of the number of bacteria added.

The same quantities of nitrifying bacteria added to different soils in some cases produced different quantities of nitrates; this may be due to differences in the chemical composition of the soils.

The same sterilized soil inoculated with cultures of different soils in some

cases produced different quantities of nitrates; this may be due to differences in the nature or activity of the organisms in the different cultures.

Soils of high nitrifying capacity after sterilization sometimes produce more nitrates, with the same quantity of inoculant, than soils of low nitrifying capacity.

The nitrite organisms may be present in sufficient number to produce appreciable amounts of nitrites from ammonium sulfate when the nitrate organisms are not sufficiently abundant or active to produce nitrates.

Nitrites may be produced from ammonium sulfate when calcium carbonate is added, though few nitrates may be produced at the same time.

The quantity of nitrites from ammonium sulfate in a sterilized soil was found to be highest when 0.1 gm. of inoculant was used to 200 gm. of sterilized soil and decreased with the amount of inoculant, until, with 2.0 gm. of inoculant, practically no nitrite remained.

Nitrite organisms are either more abundant in the soil than nitrate organisms or else they multiply more rapidly.

The total production of nitrites, as measured by the sum of the nitrous and nitric nitrogen, is not in direct proportion to the numbers of organisms added. The first addition of 0.1 gm. of culture to 200 gm. of soil produces a high quantity of nitrites plus nitrates, whereas subsequent increments produce much smaller quantities.

When field soils are compared in nitrifying power for ammonium sulfate, the differences observed may be due chiefly to differences in the numbers of nitrifying bacteria at the time the experiment was begun. Abundance or deficiency in calcium carbonate may be another important factor.

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# THE MICROFLORA OF THE ASH OF KATMAI VOLCANO WITH ESPECIAL REFERENCE TO NITROGEN FIXING BACTERIA

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The revegetation of volcanic areas or more precisely the colonization of volcanic ash which is inorganic and practically free from combined nitrogen offers opportunity for the study of many problems connected with the genesis of a soil not to be duplicated elsewhere. It was primarily with the hope of contributing to a knowledge of humus formation and of the "nitrogen cycle" that the National Geographic Society dispatched expeditions under the leadership of R. F. Griggs to the area devastated by the eruption of Katmai in 1912. But during the period covered by the five earlier expeditions, 1915 to 1919 inclusive (5, 7), the ash remained bare and nothing could be done with the main problem beyond laying the foundations necessary for understanding colonization when it should occur. The expeditions of these years, however, resulted in the discovery of the Valley of Ten Thousand Smokes and the publication of important contributions to an understanding of mineralization and volcanism.

After an interval of 11 years Griggs returned to Katmai, in 1930, again under the auspices of the National Geographic Society. This time the ash was found to be covered with a carpet of liverworts (*Jungermanniaceae*) in pure stand, as detailed elsewhere (6). Since the nitrogen content of the ash is very low, the possibility of nitrogen fixation at once suggested itself. This paper is an attempt to deal with that problem as far as it concerns nitrogen fixing microorganisms with which the liverworts might be associated.

## METHODS AND MATERIALS

Previous to the departure of the expedition in 1930, the collection of samples of ash was discussed and the decision was made to bring back samples taken aseptically which could be analyzed bacteriologically and the flora compared in a general way with that of soil. All materials taken on the expedition or brought back had to be limited because of the fact that everything had to be transported several miles on mens' backs. Therefore, instead of having large samples, we had to be content with only a few grams of materials. The con-

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tainers were small test tubes which were plugged with cotton and sterilized before leaving Washington, D. C. In obtaining the samples, a test tube with the plug removed was pushed horizontally into the perpendicular cut surface of the ash. The test tube was then sealed off in an alcohol flame. As a control as to whether sealing the tube for 2 or 3 months would have any great detrimental effect on the microflora, garden soil from near Washington was sealed in this manner and carried to Katmai and return. Other samples of ash were taken in sterile tubes and replugged. These samples, of course, soon dried out and remained air dry until plated out several months later.

When the samples were received in the laboratory in Washington, D. C., 2-gm. portions were aseptically weighed out, dilutions made in sterile tap water in steps of 10, starting with a 1 to 50 dilution. From each of these dilutions five plates were poured with each of the various media employed.

The medium used for determining the number of microorganisms present in the ash was soil extract agar. This medium is the best one available, as it allows the greatest number of microorganisms to develop when compared to other substrates (21). It should not be considered perfect, for it is known that groups of bacteria, especially the autotrophic bacteria, will develop only on special media, making a universal medium out of the question.

The soil extract is prepared by autoclaving 500 gm. of air-dry field soil and 1,500 cc. of tap water for one-half hour at 15 pounds pressure. After the soil extract has been filtered clear through paper, 0.05 per cent  $K_2HPO_4$  and 1 per cent agar are added, and the reaction is adjusted to pH 6.8, if necessary. The plates were incubated at 28°C. for 14 days.

For estimating the number of fungi in the ash samples, plates were poured with acid dextrose nitrate agar. This is prepared by adding to soil extract 0.1 per cent  $KH_2PO_4$ , 0.1 per cent  $NaNO_3$ , 2.5 per cent agar, and 1 per cent dextrose. The dextrose is added after the agar is melted and filtered through cheesecloth to remove any large particles of dirt. The reaction is adjusted to pH 4.2, by adding approximately 1 cc. each of normal HCl and  $H_2SO_4$ . Sterilization of this acid medium is accomplished by autoclaving 12 minutes at 12 pounds with the containers set in a water bath. This keeps the agar from breaking down too much and has been entirely safe as far as contaminations are concerned. The plates were incubated for 3 days at 28°C.

The medium used in pouring plates for actinomycetes consisted of soil extract to which was added 0.1 per cent  $K_2HPO_4$ , 0.1 per cent  $NaNO_3$ , 1 per cent glycerine, and 1 per cent agar. The reaction was adjusted to pH 6.8. Plates were incubated 10 days at 28°C.

Plates were also poured with Ashby's nitrogen-free agar (1) in order to see whether nitrogen-fixing bacteria were present in the ash. The ingredients of the medium were not purified by recrystallization, consequently traces of nitrogen were present, as is usually the case. These plates were incubated for 10 days at 28°C.

TABLE 1

*Plate counts of groups of microorganisms in various samples of volcanic ash and the relative occurrence of B. radiobacter among cultures isolated from Ashby's agar plates*

Per moist gram unless otherwise noted

NUM- BER	DESCRIPTION	PLATE COUNTS	FUNGI	ACTINO- MYCETES	MUCOID COLONIES	MUCOID COLO- NIES ISO- LATED	IDENTI- FIED AS <i>B.</i> <i>radiobacter</i>
1	Undisturbed ash, bare, 23	140,000	0	0	4,000	11	0
2	Fine ground ash, windblown and bare, 30	160,000	few	0	4,000	..	..
3	Windblown ash around roots of Calamagrostis, 22	70,000	0	0	20,000	10	0
4	Bright green liverwort layer, air dry, 4	65,000	50	0	3,250	8	8
5	Bright brown liverwort layer, air dry, 9	277,000	200	0	55,000	7	6
6	Black liverwort layer, sam- ple kept in dark, butyric odor, 13	2,700	50	0	200	.	.
7	Black liverwort layer bu- tyric odor, 15	1,000*	1,600	0	100*	..	.
8	1 inch below black liverwort layer; black coloration; butyric odor, 20	500,000	0	0	1,200	9	3
9	Duplicate of above, except no color and no odor, 20	900,000	450	0	1,000	.	..
10	6 inches below black liver- wort layer, 21	1,400,000	0	0	60,000	9	2
11	Top ash with dead rhizoids, 29	300,000	0	0	8,000	10	8
12	2 inches below liverwort layer at Kodiak, 31	80,000	few	0	40,000	7	3
13	Lupine root and ash, 24	570,000	120	0	400,000	8	0
14	Ash near lupine roots, 25	1,600,000	40,000	0	150,000	9	2
15	Old soil from exposed crest, 28	65,000	50	0	11,000	4	0
16	Dry liverwort in envelope, not taken aseptically	72,000	31,000	0	2,750	7	7
17	Sealed soil, carried to Kat- mai and return, 19	9,200,000	8,000	1,700,000	6,000	..	..
18	Fresh soil from same locality	19,200,000	49,000	2,750,000	6,000	..	..

\* Plates covered by fungi, accurate counts impossible.

#### NUMBERS OF MICROÖRGANISMS IN THE ASH

The plate counts on soil extract agar are given in the first column of table 1. The count of 140,000 microorganisms per moist gram of the undisturbed ash is very low if compared to a fertile soil similarly sealed and analyzed (number 17). Even lower counts than this were obtained in most of the samples of liverwort.



Of the five samples of liverwort plated out, two were taken in sterile test tubes and not sealed, the cotton plug being replaced in the tube, two were sealed and remained moist, and one was a sample brought in a package without precautions to avoid contamination by microorganisms. The numbers in the dry liverwort taken aseptically (numbers 4 and 5) were 65,000 and 277,000 whereas in the moist sealed samples (numbers 6 and 7) they were 2,700 and 1,000 or more. Since these moist sealed samples had a butyric odor it is logical to suppose that they had undergone some changes detrimental to the aerobic forms. The numbers in bulk sample number 16 agree well with those in sample 4, indicating that no contamination had occurred in the former while being transported in the paper envelope. Its dry condition would militate against any change in the flora.

Underneath the liverwort layer, larger numbers were found than were found in the liverwort layer. The sample taken 1 inch below (number 8) showed a black discoloration and had a butyric odor whereas the duplicate, number 9, showed no darkening and had no odor. The count of 900,000 in the latter is probably nearer the truth. In the sample taken 6 inches below the surface (number 10) still larger numbers were found. However, this count (1,400,000) is not as large as found in ordinary soil. The ash around lupine roots (number 14) contained about the same number of microorganisms, although the root with adhering ash contained only 570,000.

The soil, sealed and transported to Alaska and return (number 17), seems to have changed somewhat when compared with fresh soil taken from the same locality. The fresh sample, however, was taken after the return from the expedition and is hardly comparable to the sealed sample taken some months previously. Even if they were comparable, the reduction from 19,000,000 to 9,200,000 would not be very serious. It is quite possible that more change might have occurred in the sealed liverwort due to the relatively high percentage of organic matter.

#### THE NUMBERS OF FUNGI

The absence of fungi in the ash is very striking. Where liverwort is growing fungi seem to be able to exist. In number 7, the fungi overgrew all plates so that it was impossible to make anything like accurate counts of the mucoid colonies or of the total numbers. The dry bulk sample (number 16) showed by far the highest number of fungi among the liverwort samples (31,000), the other dry samples (numbers 4 and 5) containing only 50 and 200 respectively. If this were not due to chance sampling it may be that the large bulk dried out more slowly than the small samples in the test tubes thereby giving the fungi a chance to form spores. The ash near the lupine roots contained 40,000 fungi per gram whereas the lupine root with adhering ash contained only 120.

As stated in the foregoing, numbers 17 and 18 are not comparable, inasmuch as the fresh soil sample (number 18) was taken several months later than number 17. However, it is probable that some reduction in numbers took

place due to the sealing of the soil sample (number 17). But it is not probable that the absence of fungi from the ash and the low numbers in the liverwort can be due to this cause.

#### THE ABSENCE OF ACTINOMYCES IN THE ASH

No actinomycetes were found in any of the ash or liverwort samples. This probably means that conditions were not favorable for their development. It is not conceivable that inoculation of the ash had not occurred during the 18 years' exposure to the elements.

Although the actinomycete group has been studied considerably in the laboratory (23) very little is known of their physiology under natural conditions. In ordinary soil they comprise from 15 to 40 per cent of the total count depending upon the crop grown and soil treatment. The highest numbers are found under sod (23, p. 40). One would expect to find some in sample number 15, old soil from exposed crest. Their absence is inexplicable.

#### BACTERIA DEVELOPING ON NITROGEN-FREE MEDIA

The *azotobacter* group produce large, easily recognized colonies on Ashby's agar, whereas the colonies of the root-nodule bacteria (*Rhizobia*) are smaller and not distinguishable from colonies of other bacteria. Both the *Rhizobia* and these other bacteria produce more or less mucoid colonies on Ashby's agar. Their growth means that they are either using the nitrogen of the air or can grow with the very small amounts of nitrogen present in the medium as contamination. Undoubtedly many bacteria not belonging to the *azotobacter* group fix a little nitrogen when conditions are right. On the other hand, it should not be taken for granted that if colonies grow on Ashby's medium they fix nitrogen.

No colony of *Azotobacter* was seen on any of the plates made from the ash or liverwort. But since some bacteria appeared to grow on the nitrogen-free medium it was thought advisable to make isolations from the plates and to observe the cultures more closely. Accordingly several of the most vigorous growing colonies were picked from the Ashby agar plates, and inoculated into the same medium. After 2 days' growth, smears were stained by the Gram method. If the cultures were pure, transplants were made to beef extract agar, milk, gelatin, and glycerine nitrate agar.

The isolations made from the undisturbed ash (number 1, table 1) were all gram-negative small rods, growing weakly on Ashby's agar and as a flat gray growth on beef extract agar, coagulating and sometimes digesting milk. Four of the eleven liquefied gelatin. From these observations, it was concluded that nothing could be gained by further study of these cultures since none of them resembled any of the ordinary nitrogen-fixing bacteria. Similar bacteria were isolated from the windblown ash (number 3).

The observations on the eight mucoid cultures isolated from the bright green liverwort layer (number 4) showed that all of the cultures belonged to the *B.*

*radiobacter* group. Similar results were obtained from plating the sample of the bright brown layer of liverwort (number 5), the bulk sample of liverwort (number 16), and the top ash with dead rhizoids (number 11).

No isolations were made from the moist sealed liverwort samples (numbers 6 and 7) because the plates were overgrown by fungi.

It is also interesting to note that from the sample taken 1 inch below the liverwort (number 8), three of the nine isolations were *B. radiobacter*; from 2 inches below (number 12), three of the seven; and from 6 inches below (number 10), two of the nine.

It is rather surprising that no colonies of *B. radiobacter* were included in the eight mucoid colonies which were picked from the plates made from lupine roots (number 13). This organism is abundant around the roots of legumes growing in soil (20). In this case, it is possible that *B. radiobacter* was outnumbered by other bacteria on account of the decay of the roots and did not appear in such high dilutions. The fact that two of the nine colonies isolated from ash near the roots (number 14) proved to be *B. radiobacter* seems to support this assumption.

No colonies of *B. radiobacter* were found on the plates made from the old soil of exposed crest (number 15) although the number of colonies on Ashby's agar was noticeably high. The four cultures isolated were gram-positive spore formers.

Cultures of liverwort grown by one of us (R. F. G.) on nitrogen free agar or in solution always have *B. radiobacter* as a contamination. The growth of the bacterium is scant and often not discernible in solutions. Whether its presence on the rhizoids is significant can not be stated at this time.

*B. radiobacter* is a soil bacterium closely related to the nodule-bacteria (*Rhizobia*), to the crown gall organism (*Phylomonas tumefaciens*), and to *Aerobacter aerogenes*. It has been found to be more numerous under sod than in bare soil (16), and is generally more numerous in soil on which legumes are growing (20), although certain non-legumes, as rape (22), seem to increase the number also.

The function of *B. radiobacter* in the soil has not been definitely determined. In pure cultures in the laboratory, small amounts of nitrogen have been fixed in a number of cases (3, 4, 9, 10, 11, 13, 14, 19) whereas in other cases (2, 18, 19) nitrogen was not fixed or was fixed by some cultures and not by others. Some have even reported a loss of nitrogen from the culture solution, especially if some nitrate is present (2, 3, 4, 12, 18). This has led to the conclusion that *B. radiobacter* has three distinct physiological reactions, viz., nitrogen fixing, nitrate assimilating, and nitrate reduction.

#### NITROGEN FIXATION EXPERIMENTS

Two flasks containing 50 cc. of Ashby's solution and two flasks of Ashby's solution with 1 per cent dextrose substituted for mannite were inoculated with about half a gram of sample numbers 1, 3, 6, 9, 10, 11, 14, 15, 17 and with

a control fresh soil sample. No visible growth occurred in either solution when inoculated with the samples of ash or liverworts. Sample number 17 and the control soil gave a good growth and each had an azotobacter film. The nitrogen fixed in Ashby's solution by number 17, the sealed soil sample, and by the fresh control soil was 4.7 and 3.4 mgm., respectively; when dextrose was substituted for mannite in that solution it was 3.8 and 2.6 mgm., respectively. The flasks inoculated with number 6, the black liverwort layer, and those inoculated with number 11, ash with dead rhizoids were also analyzed for total nitrogen. As no growth took place in these flasks, no fixation was anticipated and none was found.

In view of the fact that *B. radiobacter* has been reported as fixing nitrogen, it was thought advisable to try pure cultures isolated from a liverwort sample. Since this organism apparently did not grow in Ashby's solution, as has been noted, soil extract with 0.05 per cent  $K_2HPO_4$  and 1 per cent mannite was used for this test. The amount of growth which developed in 15 days was not very great, although there was a definite development of the organism. Analysis for total nitrogen gave 0.1, 0.17, and 0.15 mgm., above the control per 100 cc. solution. This apparent fixation probably can be explained by the inaccuracies of the modified Kjeldahl method in such work (8) or can be attributed to a loss of nitrogen from the control medium (15) or to some other cause.

Of course, the possibility should not be overlooked that under different cultural conditions nitrogen might be fixed by pure cultures or that nitrogen might be fixed by the liverwort and *B. radiobacter* in symbiosis.

The nitrogen fixing microorganisms occurring in disintegrating lava on the slopes of Vesuvius were studied by Riccardo (17). Pure cultures of a *Mycoderma* showed a fixation of nitrogen ranging from 1.8 mgm. to 3.4 mgm. per 100 cc. solution in five of nine flasks, the other four flasks failing to show any increase in nitrogen. Only one of the three flasks inoculated with an organism identified as *B. amylobacter* showed any nitrogen fixation, whereas seven of the eight flasks inoculated with a bacterium which he believed to belong to the genus *Azotobacter* showed a gain of nitrogen ranging from 1.1 mgm. to 2.6 mgm. per 100 cc. of solution.

Although the lavas studied by Riccardo were still devoid of higher plants, they were considerably disintegrated from exposure to the weather for about 250 years. Inasmuch as the ash of Katmai was only 18 years old when sampled, it is obvious that the conditions obtaining there are not comparable with those in the weathered lava.

#### SUMMARY

Samples of bare volcanic ash collected 18 years after the eruption of Mt. Katmai contained no fungi or actinomycetes. The number of bacteria was about 150,000 per gram and comprised none of the organisms commonly known to be able to fix nitrogen.

Samples of ash on which a layer of various species of liverworts (*Jungerman-*

*niaceae*) were growing were plated out with the following results: Fungi were usually present in small numbers; no actinomycetes were found; the numbers of bacteria found in the surface layer varied from 65,000 to 300,000, 1 inch below the layer 900,000, and 6 inches below, 1,400,000.

*B. radiobacter* was readily isolated from Ashby agar plates made from the liverwort layer. It was also found underneath the liverwort but to a less degree. No culture of *Azotobacter* was obtained nor was there any development of azotobacter slime in Ashby's solution.

Nitrogen fixing experiments using Ashby's solution and inoculated with the ash or liverwort layer, failed to show any fixation, whereas a fresh soil and a sample sealed like the ash samples both gave a fair fixation and the typical azotobacter pellicle.

Pure cultures of *B. radiobacter* grown in a soil extract mannite solution also failed to fix appreciable amounts of nitrogen.

Therefore it is concluded that if fixation of nitrogen occurs in volcanic ash, it can not be attributed to the microflora.

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## NITROGEN-FIXERS OF LEACHED ALKALI SOILS<sup>1</sup>

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Most Utah soils studied, if incubated with optimum moisture and an appropriate carbohydrate, fix nitrogen (1). Many of these soils on incubation, without the addition of a carbohydrate, gain in nitrogen. A few of the soils studied, if mixed with plant residues and kept in a greenhouse, fix appreciable quantities of nitrogen (4). The quantity of nitrogen gained varies with the specific soils, the plant residues added, and the specific salts within the soil. The microorganisms which take part in the nitrogen fixation are quite resistant to so-called "alkali salts" (2), and some "alkali salts" accelerate the rate at which the soils gain nitrogen. A soil may gain considerable nitrogen under these specified conditions and still not give a typical *Azotobacter* membrane when inoculated into Ashby media (3). This paper considers some comparatively active nitrogen-fixing microorganisms which have been obtained from three "alkali soils:" (a) the college farm soil which had been rendered unproductive by the addition of 0.66 per cent each of sodium chloride, sodium sulfate, and sodium carbonate; (b) a Corinne soil which, before being leached, was so high in soluble salts, principally chlorides, that it was barren; and (c) a Richland Acres soil which contained sufficient soluble salts, principally sulfates, to prevent the growth of most plants. Each of these soils had been leached with water until the major portion of the soluble salts was removed before the microorganisms were isolated. After being leached, the soils had produced small crops of crimson clover and barley (6).

The pure cultures were obtained from Ashby agar by repeated dilutions, plating, and microscopic examinations. Their nitrogen-fixing ability was determined by incubation in sterile soil to which an appropriate carbohydrate had been added. The stock cultures were kept on the soil extract, Ashby agar. This soil extract was prepared by extracting 2 gm. of soil with 100 cc. of distilled water. The microorganisms were studied both morphologically and physiologically according to the descriptive chart prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists

<sup>1</sup> Contribution from department of bacteriology and chemistry, Utah Agricultural Experiment Station.

<sup>2</sup> Bacteriologist and graduate student, respectively.

The authors wish to express their appreciation to Milton Nelson for assistance in the nitrogen determinations.

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(9). A careful study of the literature and related organisms makes it highly probable that these are new species or at least new varieties. However, we are merely designating the organisms by a number and giving the main morphological and physiological characteristics.

*Bacterium 16b.* Gram-positive, non-spore-forming rods 0.6 to 0.7 by 1 to 1.7  $\mu$ , occurring singly.

*Gelatin colonies:* Rapid, punctiform, cream, entire, coarsely granular growth.

*Gelatin stab:* Liquefaction crateriform, filiform with good surface growth, medium unchanged.

*Agar colonies:* Rapid, circular, smooth, raised, entire, finely granular.

*Agar slant:* Abundant, light yellow, glistening, raised, contoured, butyrous growth.

*Broth:* Slight clouding, no sediment.

*Litmus milk:* Neutral with slow peptonization. Litmus reduced.

*Potato:* Abundant, yellow-tan growth, potato darkened.

Produces indole, reduces nitrates with the formation of gas. Produces ammonia from peptone, hydrolyzes starch, produces acid on glucose and sucrose but not on lactose. Aerobic. Habitat, College Farm soil.

*Micrococcus 17b.* Gram-negative, non-motile cocci 0.8 to 1  $\mu$ , occurring in sheets and in clusters.

*Gelatin colonies:* Slow, punctiform, raised, entire, finely granular, cream-colored growth.

*Gelatin stab:* Villous, no liquefaction.

*Agar colonies:* Rapid, circular, punctiform, smooth, raised, entire, finely granular light pink growth.

*Agar slant:* Abundant, echinulate, raised, glistening, contoured, opaque, butyrous growth.

*Broth:* No surface growth, slight clouding with scanty flocculent sediment.

*Litmus milk:* No change.

*Potato:* No growth.

Reduces nitrates and produces acid on lactose and glucose but not on sucrose. Produces ammonia in peptone. Aerobic. Habitat, College Farm soil.

*Bacillus 6b.* Gram-positive rods 0.9 to 1.2 by 1.9 to 3.3  $\mu$ , occurring in chains. Motile by means of monotrichous flagellum. Spores terminal, spherical 0.5 to 0.7  $\mu$ . Gram-positive

*Gelatin stab:* Liquefaction crateriform.

*Agar colonies:* Rapid, circular, smooth, raised, entire, finely granular, pink colonies.

*Agar slant:* Abundant, peach-colored, glistening, opaque, contoured, butyrous growth.

*Broth:* No clouding, no surface growth.

*Litmus milk:* Slightly acid.

*Potato:* No growth.

Hydrolyzes starch. Produces indole. Reduces nitrates but does not produce acid on glucose, lactose, or sucrose. Produces ammonia in peptone solution. Aerobic. Habitat, College Farm soil.

*Bacterium 119b.* Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.7 by 1.5 to 1.9  $\mu$  occurring in clusters.

*Agar slant:* Abundant, echinulate raised, glistening, smooth, opaque, butyrous growth.

*Gelatin stab:* Liquefaction crateriform.

*Broth:* No clouding or surface growth.

Does not produce indole nor reduce nitrates. Produces ammonia in peptone. Hydrolyzes starch, produces acid on glucose, but not on lactose or sucrose. Aerobic. Habitat, Corinne alkali soil.

*Micrococcus 115a.* Gram-positive, non-spore-forming cocci 0.5 to 0.8  $\mu$ , occurring in pairs and clusters.

*Gelatin colonies:* Slow, punctiform, raised amorphous with entire edges.

*Gelatin stab:* Liquefaction crateriform.

*Agar colonies:* Rapid, circular, smooth, raised, amorphous with entire edges.

*Agar slant:* Abundant, echinulate, convex, glistening, smooth, opaque, butyrous growth.

*Broth:* Slight clouding.

*Litmus milk:* Alkaline slow peptonization.

*Potato:* Abundant, tan, spreading growth.

Produces indole, does not reduce nitrates but produces ammonia from peptone and urea. Hydrolyzes starch but does not produce acid from dextrose, sucrose, or lactose. Aerobic. Habitat, Corinne alkali soil.

*Archromobacter 11a.* Gram-negative, non-motile rods 0.5 to 0.6 by 2.4 to 6  $\mu$ , occurring singly and in clusters.

*Gelatin colonies:* Slow, small, punctiform, raised, entire finely granular with cup-like liquefaction.

*Gelatin stab:* Slight liquefaction. Good surface growth. Medium unchanged.

*Agar colonies:* Rapid, punctiform, smooth, raised, entire, coarsely granular colonies.

*Agar slant:* Milky, abundant, echinulate, glistening, contoured, opaque, butyrous growth.

*Broth:* No clouding, no pellicle formation.

*Litmus milk:* Alkaline with slow peptonization.

*Potato:* Slight, raised, milky growth.

Produces indole, does not reduce nitrates nor ammonify peptone. Aerobic. Habitat, College Farm soil

*Micrococcus 114d.* Gram-positive, non-motile, non-spore-forming cocci 1.2 to 1.6  $\mu$ , occurring in pairs and clusters

*Gelatin colonies:* Slow, irregular, raised, coarsely granular with lobate edges and saucer liquefaction.

*Gelatin stab:* Liquefaction saccate. Reddish brown in color.

*Agar colonies:* Rapid, punctiform, smooth, raised, finely granular with entire edges.

*Agar slant:* Moderate, raised, glistening, opaque, cream-colored growth.

*Broth:* Slight clouding and very scanty sediment.

Does not produce indole, reduce nitrates nor hydrolyze starch. Produces ammonia in peptone. Aerobic. Habitat, Corinne alkali soil.

*Bacterium 202b.* Gram-negative, non-motile, non-spore-forming rods 0.5 to 0.7 by 1.2 to 1.9  $\mu$ , occurring singly and in clusters.

*Gelatin colonies:* Slow, punctiform, cream-colored.

*Gelatin stab:* Liquefaction napiform.

*Agar colonies:* Rapid, circular, smooth, raised with entire edges.

*Agar slant:* Abundant, glistening, raised, opaque growth.

*Broth:* Moderate clouding with flocculent surface growth and sediment.

*Litmus milk:* Alkaline with slow peptonization, litmus reduced.

*Potato:* Abundant tan growth. Medium darkened.

Produces indole. Does not reduce nitrates. Hydrolyzes starch. Produces acid in dextrose media. Aerobic. Habitat, Richland Acres alkali soil.

*Actinomyces 227a.* Mycelium straight with little branching.

*Gelatin colonies:* Rapid, raised filamentous.

*Gelatin stab:* Liquefaction crateriform.

*Agar colonies:* Rapid, irregular, raised undulate, radially ridged, coarsely granular.

*Agar slant:* Abundant, echinulate, dull, raised rugose, translucent brown, butyrous growth.

*Broth:* Slightly cloudy, with abundant flocculent sediment and surface growth.

*Litmus milk:* Acid with peptonization.

*Potato:* Moderate white growth. Potato darkened around growth.

Produces indole. Reduces nitrates. Produces acid on glucose. Hydrolyzes starch and actively decomposes cellulose. Aerobic. Habitat, Richland Acres alkali soil.

*Bacterium 16e.* Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.6 by 0.7 to 0.9  $\mu$ , occurring singly and in clusters.

*Gelatin stab:* Liquefaction crateriform.

*Agar colonies:* Rapid, circular, smooth, flat, entire, ivory-colored, finely granular.

*Agar slant:* Abundant, echinulate, raised, glistening, ivory-colored growth.

*Broth:* Moderate clouding, no pellicle formation.

*Litmus milk:* Slightly acid with slow peptonization.

*Potato:* Light creamish tan, abundant growth. Potato darkened.

*Produces indole.* Slowly ammonifies peptone but does not reduce nitrates nor hydrolyze starch. Produces acid on glucose but not on sucrose or lactose. Aerobic. Habitat, College Farm soil.

*Micrococcus 4.* Spheres 0.7 to 1.2  $\mu$ , occurring in clusters and pairs. Gram-negative.

*Gelatin colonies:* Very small, punctiform, slow to develop, raised, entire, finely granular colonies.

*Gelatin stab:* Liquefaction crateriform slow surface growth.

*Agar colonies:* Rapid, circular, smooth, raised, entire, amorphous.

*Agar slant:* Abundant echinulate, raised, glistening, opaque growth.

*Broth:* Slightly clouded, no surface growth.

*Litmus milk:* Alkaline with slow peptonization.

*Potato:* Abundant light tan growth.

Does not produce indole nor reduce nitrates. Hydrolyzes starch but does not produce acid on glucose, sucrose, or lactose. Produces ammonia in peptone solution. Aerobic. Habitat, College Farm soil.

The nitrogen-fixing powers of the various organisms were determined as follows: One-hundred-gram portions of soil pulverized and passed through a sieve with round holes 1 mm. in diameter were weighed into 500-cc. Erlenmeyer flasks, the moisture made up to 20 per cent and stoppered with cotton. They were then autoclaved for 2½ hours at 120°C., after which 10 cc. of sterile distilled water containing 1.0 gm. of the specific carbohydrate was added to each flask. Bacteriological tests were made on the soils to make certain they were sterile, after which they were inoculated in triplicates with the specific organism to be tested. Sterile checks were run in both these and all other series reported. All were incubated for 5 weeks at 28°C., the moisture content being maintained with sterile distilled water at approximately 26 per cent. At the end of 5 weeks the samples were dried at 120°C., pulverized, sieved as before, and the total nitrogen was determined by Official Gunning method (8, p. 8). Blanks were run on all chemicals and a high grade of sodium hydroxide was used, thus preventing frothing and loss of nitrogen. The results, as reported in table 1, are the average of six or more closely agreeing determinations. They represent the nitrogen gains in milligrams for 10 gm. of soil. The errors due to analyses have been calculated according to the formula,  $P.E. = .67 \sqrt{\frac{\sum d^2}{n^2}}$ ; where the value is  $\pm .04$  or less it is reported as  $\pm 0.0$ , where  $\pm .05$  to  $\pm 0.1$  it is reported as  $\pm 0.1$ .

The 11 organisms fixed appreciable quantities of nitrogen, the quantity varying with the specific organism and the carbohydrate at its disposal. The average fixation was highest on sucrose and arabinose. Usually only small

quantities of nitrogen were fixed where xylose, maltose, or dextrose furnished the energy. Starch and inulin are quite acceptable sources of energy to the majority of the microorganisms. Mannite and sodium lactate are only fair sources of energy. It is interesting to note that Löhnis and Pillai (7) found dextrose to be only one-half as valuable to *Azotobacter* as is sucrose. They found starch low in value to *Azotobacter*, whereas in the experiment reported it is quite acceptable. These organisms appear to be from one-third to two-thirds as efficient in the fixation of nitrogen on the various carbohydrates as Löhnis and Pillai found the *Azotobacter*.

The soil in which these organisms have been cultured contains, as determined by the official Kjeldahl method, 0.132 per cent nitrogen; when determined by the absolute, or cupric oxide method (8, p. 9), however, it yielded 0.159 per cent nitrogen, which shows that only 83 per cent of the nitrogen carried by this soil is obtained by the Kjeldahl method. Therefore, each 100 gm. of soil contains 27 mgm. of nitrogen which is not determined by the latter method. Consequently, it may be argued that the gains in nitrogen obtained when this soil is sterilized, inoculated with the specific microorganism, and then incubated, result from the transforming of the non-determinable pyrrole nitrogen and the small quantity of nitrates present into nitrogen compounds of such a nature that it can be determined by the Kjeldahl method. If this were true, the gains in nitrogen would be only apparent and the organisms would be transforming nitrogen compounds but would not be fixing atmospheric nitrogen. In order to test this supposition 10 of the organisms were inoculated separately into sterile sucrose-containing soil. These were incubated for 5 weeks at 28–30°C. with optimum moisture content. Along with these were incubated sterile sucrose-containing soil. At the end of the incubation period the soils were dried and the nitrogen determined by the absolute method.

The average fixation in this set (table 2) was slightly higher than in the former. The order of efficiency changes, but in every case except that of *Micrococcus* 17b there were appreciable gains of nitrogen. Nor could it be argued that this nitrogen came from combined nitrogen, ammonia, nitrous and nitric acid, taken from the air since beside each inoculated flask was a similar sterile flask which was used for a check.

The observed gains in nitrogen are small yet significant, as is evident from the following facts: (a) Extreme care has been taken in the nitrogen determinations. Blank checks were run on all chemicals and determinations made on sterile soil containing the same quantity of water and carbohydrate and incubated alongside of the inoculated soil; the reported gains are usually considerably more than three times the experimental error. (b) More than 200 uniformly positive results have been obtained on these organisms when grown on various carbohydrates. (c) When the soils from which these organisms were obtained were kept under carefully controlled conditions in a greenhouse with optimum moisture and an appropriate source of energy, carbohydrate, or plant residue, for from 3 to 5 years, they gained appreciable quantities of

TABLE 1  
 Milligrams of nitrogen fixed in 10 gm. of soil containing various carbohydrates and inoculated with pure cultures of nitrogen fixers

	SUCROSE	ARABIN- NOSE	INULIN	STARCH	LACTOSE	GALAC- TOSE	MANNITE	SODIUM LACTATE	XYLOSE	MALTOSE	DEXTROSE
<i>Bacterium</i> 16b . . . . .	1.0±0.1	1.2±0.2	0.6±0.0	0.8±0.0	0.8±0.1	1.0±0.0	0.1	0±0.2	0.4±0.0	0.2±0.2	-0.2±0.2
<i>Micrococcus</i> 17b . . . . .	0.8±0.2	0.6±0.0	0.4±0.1	0.2±0.1	0.4±0.2	0.4±0.0	0.0	4±0.2	0.8±0.0	0.6±0.0	0.4±0.0
<i>Bacillus</i> 6b. . . . .	0.2±0.0	0.2±0.0	0.8±0.0	0.1	0.6±0.0	0.6±0.2	0.2	0±0.0	0.6±0.0	0.2±0.0	0.4±0.0
<i>Bacterium</i> 119b. . . . .	0.6±0.0	0.6±0.0	0.6±0.1	0.8±0.1	0.6±0.0	0.8±0.2	0.6	0±0.1	1.2±0.1	0.2±0.1	0.0±0.0
<i>Micrococcus</i> 115a. . . . .	1.2±0.2	0.8±0.1	0.4±0.1	0.4±0.0	0.6±0.1	0.4±0.1	0.4	0±0.1	0.6±0.2	0.6±0.2	0.4±0.1
<i>Achromobacter</i> 11a. . . . .	0.6±0.2	0.6±0.1	0.6±0.1	1.0±0.2	0.8±0.0	0.8±0.1	0.2	0±0.2	0.6±0.1	0.4±0.2	0.4±0.1
<i>Micrococcus</i> 114d. . . . .	1.0±0.0	1.2±0.2	0.8±0.0	0.6±0.3	0.6±0.0	0.2±0.1	0.2	0±0.1	0.6±0.0	0.0±0.2	0.4±0.1
<i>Bacterium</i> 202. . . . .	1.0±0.1	0.8±0.0	0.8±0.1	0.6±0.1	0.6±0.2	0.4±0.0	0.6	0±0.2	0.2±0.2	0.2±0.0	0.0±0.1
<i>Actinomyces</i> 227a. . . . .	0.6±0.1	1.0±0.1	0.6±0.0	0.4±0.0	0.6±0.1	0.4±0.0	0.1	0±0.0	0.0±0.1	0.0±0.1	0.2±0.1
<i>Bacterium</i> 16c. . . . .	0.8±0.1	1.0±0.0	0.4±0.0	0.8±0.0	0.8±0.1	0.6±0.1	0.6	0±0.2	0.0±0.0	0.2±0.0	-0.4±0.1
<i>Micrococcus</i> 4. . . . .	0.6±0.2	0.2±0.1	0.6±0.0	0.4±0.0	0.8±0.1	0.6±0.1	0.6	0±0.1	0.8±0.0	0.2±0.1	0.4±0.2

nitrogen. Yet some of these soils do not harbor known species of *Azotobacter*, nor have we been successful after long and careful search in finding known species of nitrogen-fixers in the soil. It is not uncommon to find soils devoid of *Azotobacter* which gain nitrogen just as rapidly as other soils containing *Azotobacter*. Therefore, we have reached the conclusion that the increase of nitrogen observed in these soils under field and greenhouse conditions is due to the cumulative action of numerous microorganisms of low nitrogen-fixing efficiency and not to a few extremely active species.

TABLE 2

*Nitrogen fixed in 10 gm. of soil containing 1 per cent of sucrose during an incubation period of 5 weeks*

Nitrogen determined by the absolute combustion method

MICROORGANISMS	NITROGEN FIXED IN 10 GM. SOIL
	mgm.
<i>Bacillus</i> 6b .	1.4±0.2
<i>Actinomyces</i> 227a . . .	1.4±0.1
<i>Micrococcus</i> 115a . . . . .	1.2±0.1
<i>Micrococcus</i> 4 . . . . .	1.2±0.1
<i>Bacterium</i> 16b . . . . .	1.0±0.0
<i>Micrococcus</i> 114d . . . . .	1.0±0.1
<i>Bacterium</i> 16e . . . . .	0.8±0.1
<i>Achromobacter</i> 11a . . . . .	0.8±0.1
<i>Bacterium</i> 202 . . . . .	0.8±0.1
<i>Micrococcus</i> 17b . . . . .	0.2±0.2

## SUMMARY

Eleven microorganisms were obtained from leached alkali soils and studied in pure cultures; all of these possess the power of fixing nitrogen when cultured in soil with an optimum moisture content and appropriate carbohydrates. The quantity of nitrogen fixed varied with the specific microorganisms and the carbohydrate added to the soil. Sucrose, arabinose, inulin, starch, lactose, galactose, mannite, sodium lactate, xylose, maltose, and dextrose were all used by some of the organisms, the efficiency varying in the inverse order from which they are named. Xylose, maltose, and dextrose were poor sources of energy. Some of the organisms fixed as high as 1.4 mgm. of nitrogen in 10 gm. of soil. This compares favorably with fixations made by *Azotobacter*.

The possibility of the noted gains of nitrogen coming from pyrrole compounds or from combined nitrogen of the air are considered and found not to be the source of the gains.

The soils from which these microorganisms were obtained when kept in pots under greenhouse conditions with optimum moisture content gain appreciable quantities of nitrogen, most of which has been attributed to gains from atmospheric sources (4), and it is believed that the described organisms play a major rôle in the observed phenomenon.

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## PLATE 1

## MICROORGANISMS FROM LEACHED ALKALI SOIL

FIG. 1. *Bacterium* 16b.

FIG. 2. *Micrococcus* 17b.

FIG. 3. *Bacillus* 6b.

FIG. 4. *Bacterium* 119b.

FIG. 5. *Micrococcus* 115a.

FIG. 6. *Achromobacter* 11a.

FIG. 7. *Micrococcus* 114d.

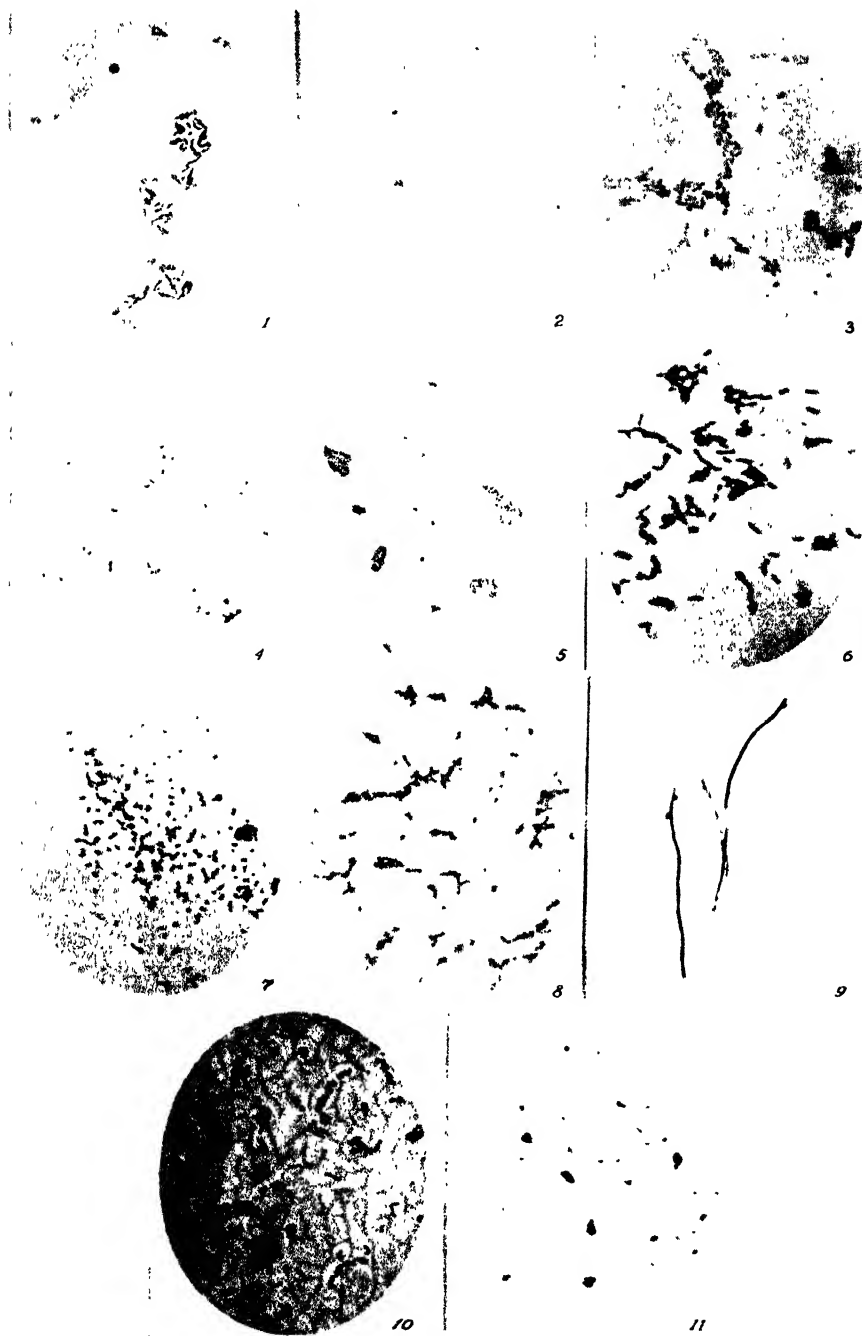
FIG. 8. *Bacterium* 202.

FIG. 9. *Actinomyces* 227a.

FIG. 10. *Bacterium* 16e.

FIG. 11. *Micrococcus* 4.

All slides were made from 3-day-old cultures, grown on nutrient agar P.H. 7.4; stained with carbol fuchsin, magnification 1100X.



FIGS. 1-11





# EFFECT OF EXCHANGEABLE BASE AND SOIL TREATMENTS ON PHOSPHORUS SOLUBILITY<sup>1</sup>

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From farm practice and experimental work it appears that the availability of phosphorus to plants, when applied as a fertilizer, varies greatly with the soil and possibly other factors. The literature contains many conflicting statements concerning phosphorus solubility and availability. The concensus of opinion seems to be that in acid soils the phosphorus is combined with iron and aluminum, and as a result of the insolubility of these phosphates the acid soils are likely to be high in total but low in soluble phosphorus. On the other hand, in neutral or basic soils the phosphorus will combine with calcium or magnesium and thus be more soluble and more available.

Comber (4) made the concise statement, "The removal of certain anions, such as phosphate ions from solution is a consequence of the exchange of bases between the soil and the solution." Double exchange may produce either a soluble or insoluble phosphate salt. Nemec and Gracinin (9) cited many conflicting statements on the resorption of phosphorus from soils, and concluded that increased adsorption of phosphorus under the influence of calcium carbonate had been demonstrated only on acid soils; and further that soils with adequate supplies of calcium show a depression in the resorption of phosphorus when treated with calcium carbonate.

Nemec (8) reported that soils lacking calcium and containing soluble iron do not respond to phosphorus fertilization even when they may be deficient in phosphorus. Soils with adequate calcium should have had more available phosphorus than soils lacking in calcium. Smirnov (11) found that saturating a degraded chernozem with Na, K, or  $\text{NH}_4$  increases the amount of soluble phosphorus. Spurway (12), on treating a soil with  $\text{KCl}$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$  to exchange the bases, found that potassium treatments always, and the magnesium treatments generally, increase the solubility of the phosphorus, whereas the calcium treatments increase the solubility in acid soils and decrease it in neutral and alkaline soils.

Stephenson and Powers (13) found that applications of sulfur to soils increase the acidity and decrease the water-soluble phosphorus. Others have reported

<sup>1</sup> Contribution No. 169, department of chemistry.

<sup>2</sup> It is with pleasure that the cooperation of W. L. Latshaw in obtaining the data in table 1 is acknowledged.

that increased acidity causes decreased phosphorus solubility. Askinazi and Yarusov (2) showed by weak acid extractions, that liming increases the solubility of  $P_2O_5$  in podzol soils. Demolon and Barbier (5) found that  $P_2O_5$  is precipitated in an alkali soil lacking  $CaCO_3$  but containing Ca, and in acid soils by means of iron sesquioxide. They also reported that liming acid soils without excess rather than acidifying increases the  $P_2O_5$  mobility. Witryn (15) concluded that alkali materials increase the solubility of ferric and aluminum phosphates, and in acid soils liming increases the solubility of phosphates by changing them to calcium phosphate. Antonov (1) reported that applications of CaO increase the pH values and water-soluble phosphorus in certain soils.

Kappen and Bollenbeck (7) found that in acid soils of the same pH values, phosphorus solubility is greater when the acidity is due to neutral salt decomposition rather than to hydrolytic acidity.

No thorough review of the literature on this problem is necessary, as this has been done by others, notably Williams (14). The purpose of this research has been to investigate the rôle that exchangeable bases play in phosphorus solubility with and without the application of fertilizers and soil amendments.

#### SOILS USED AND TECHNIQUE EMPLOYED

The soil used in this work was Cherokee loam, obtained from Cherokee County, Kansas. This is a mineral soil which naturally responds to phosphate fertilization. Samples of the soil were prepared by replacing the exchangeable cations with  $Al^{+++}$ ,  $Fe^{+++}$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $H^+$ ,  $K^+$ , and  $NH_4^+$ . This was accomplished by successive leachings of the soil with normal solutions of the various chlorides except hydrogen where a twentieth normal solution was used. The soils were treated until the leachings contained no  $Ca^{++}$ . An untreated portion of soil was also retained for use. These soils will be referred to as aluminum, iron, calcium, magnesium hydrogen, potassium, ammonium, and untreated soils.

Gedroiz (6) states that exchangeable calcium is held more tenaciously than the other bases; so it is assumed that when the calcium is replaced, equilibrium is reached. Replacement in the soil treated with calcium chloride was judged to be complete when the magnesium chloride treated soil had reached equilibrium. After total replacement had been accomplished the soils were repeatedly washed with water until the soluble chlorides were removed or reduced to such a low concentration that sedimentation would not occur rapidly. By washing with water alone, the soils treated with iron and aluminum were completely freed from soluble chlorides. The soils that had been treated with potassium, ammonium, hydrogen, calcium, and magnesium chlorides, after being washed with water were then washed with 80 per cent alcohol. By the alcoholic washings the calcium, magnesium, hydrogen, and ammonium soils were entirely freed from soluble chlorides. The potassium soil was prac-

tically freed from soluble chlorides, for the last wash recovered before sedimentation failed to occur contained only a very few parts of chloride per million. The soils thus prepared were treated with various quantities of  $\text{CaH}_4(\text{PO}_4)_2$ ,  $\text{CaCO}_3$ ,  $\text{CaCl}_2$ , and  $\text{KCl}$ , applications being at a definite rate per acre on the basis of 2,000,000 pounds of soil per acre, which will be spoken of as fertilizer treatments. The composition of the soils treated with several bases is given in table 1.

The soluble phosphorus was determined by the coeruleomolybdate method of Deniges as adapted by Atkins (3). The phosphorus solution was obtained by shaking the soil with water and then centrifugalizing the suspension, which was then passed through clay filters, the phosphorus being determined in the filtrate. To overcome the retention of phosphorus by the clay filter, which Parker (10) and others found to occur, the soil particles were rinsed from the filter, and then 45 cc. of 0.05 *N* HCl was passed through the filter, the phosphate being determined in the mixture of filtrates. Before the filters were

TABLE 1  
*Composition of Cherokee loam treated with various bases*

BASE SOIL WAS TREATED WITH	PERCENTAGE COMPOSITION							
	$\text{SiO}_2$	$\text{P}_2\text{O}_5$	$\text{Fe}_2\text{O}_3$	$\text{Al}_2\text{O}_3$	$\text{CaO}$	$\text{MgO}$	$\text{K}_2\text{O}$	$\text{NH}_4$
Fe . . . . .	82 35	0 11	3.81	6 93	0.21	0 54	0.68	0.03
Al . . . . .	82 20	0 11	2.62	8 12	0.23	0.54	0.68	0.03
Ca . . . . .	82 42	0 11	2.63	7.52	0 58	0.56	0.70	0.03
Mg . . . . .	82 20	0 11	2.93	?	0 20	0 99	0.70	0.03
$\text{NH}_4$ . . . . .	82 12	0 10	2.93	7.34	0.19	0.55	0 62	0.13
K . . . . .	82.41	0 11	2.85	7.12	0.21	0.73	1.18	0.03
H . . . . .	82.22	0.11	2.84	6 97	0 25	0 60	0.72	0.03
Untreated soil . . . . .	82.19	0 11	3 08	7.53	0 45	0 87	0.73	0.03

used a second time they were ignited and washed with 50 cc. of 0.05 *N* HCl, followed by 50 cc. of water. This method was found to give consistent results when solutions of known concentration were used. New filters were always washed with acid until the filtrate would give no blue color with Atkin's method, indicating phosphate or excessive silica. By following the procedure described in the foregoing and working with solutions of known phosphate content, it was established that no appreciable amounts of phosphorus were lost or gained as a result of absorption. Satisfactory results could be obtained regardless of the previous history of the filters.

#### DATA OBTAINED

In determining the soluble phosphate content of the soils, 25 gm. was weighed into a 500-cc. sterilizer bottle and 67.5 cc. of carbon dioxide free water was added. The bottles were shaken 4 hours on a horizontal shaker and then centrifugalized. Of the resultant suspension 50 cc. was filtered for the phos-

phorus determination, and the pH was determined on the remainder. Because of the small amount of suspension which was available for the pH determination, usually only about 1 cc. whereas the soil held about 16 cc., it was sometimes hard to obtain checks on duplicate samples and in certain cases the pH values are open to some question. In a few cases the pH data reported have been obtained in part by considering the results in other tables where

TABLE 2

*Effect of  $\text{CaH}_4(\text{PO}_4)_2$  on phosphorus solubility and pH*

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

$\text{CaH}_4(\text{PO}_4)_2$ POUNDS PER ACRE	BASE SOIL WAS TREATED WITH															
	H		Fe		Al		None		Mg		Ca		$\text{NH}_4$		K	
	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH
0	0.20	2.97	0.19	3.64	0.15	3.88	0.21	4.77	0.19	5.19	0.20	5.17	0.62	5.97	0.59	6.00
100	0.24	2.97	0.20	3.64	0.16	3.85	0.22	4.77	0.20	5.22	0.24	5.24	1.65	6.02	2.58	5.80
250	0.40	2.97	0.20	3.64	0.17	3.85	0.26	4.77	0.41	5.21	0.77	5.30	3.65	6.07	6.35	6.00
500	0.72	2.97	0.20	3.64	0.16	3.61	0.78	4.82	1.56	5.05	1.64	5.34	4.82	6.00	8.17	5.94
750	1.36	2.97	0.20	3.64	0.17	3.58	2.14	4.62	2.02	5.15	1.62	5.39	7.02	6.07	9.31	5.84
1,000	2.36	2.97	0.20	3.64	0.17	3.56	4.13	4.75	2.19	5.12	2.21	5.40	9.35	6.02	10.32	5.77
1,250			0.23	3.49	0.46	3.99										
1,500			0.36	3.46	0.73	3.99										
2,000			0.48	3.36	0.86	4.04										

TABLE 3

*Effect of  $\text{CaCO}_3$  on phosphorus solubility and pH*

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

$\text{CaCO}_3$ , TONS PER ACRE	BASE SOIL WAS TREATED WITH															
	H		Fe		Al		None		Mg		Ca		$\text{NH}_4$		K	
	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH	$\text{P}_2\text{O}_5$	pH
0	0.20	3.05	0.19	3.67	0.15	3.88	0.26	4.85	0.19	5.15	0.21	5.17	0.55	5.80	0.49	6.03
½	0.21	3.15	0.19	3.82	0.15	4.35	0.27	5.20	0.19	5.72	0.20	5.81	0.44	6.39	0.47	6.56
1	0.21	3.36	0.20	4.00	0.15	4.54	0.27	5.55	0.19	6.23	0.20	6.55	0.39	6.69	0.44	6.82
2	0.18	3.77	0.21	4.22	0.21	4.77	0.29	6.21	0.18	6.92	0.19	7.05	0.32	7.08	0.40	7.16
5	0.15	4.72	0.26	5.59	0.21	6.10	0.34	6.99	0.22	7.41	0.16	7.15	0.30	7.64	0.33	7.61
10	0.14	6.75	0.27	7.25	0.23	7.16	0.33	7.23	0.25	7.47	0.17	7.41	0.27	7.77	0.32	7.77

duplicate determinations were made. In the case of the  $\text{NH}_4$  and K treated soils, with little or no fertilizer, it was hard to make the phosphorus determinations because of the masking of the blue color by dissolved organic matter. These determinations have been reported as found but have a larger probable error than the other determinations. By comparing the data in the first lines of tables 2, 3, 4, and 5 their reliability can be ascertained.

The effect of various fertilizers and soil amendments on phosphorus solubility, when the exchangeable bases varied in the soil, was determined and the data are reported in tables 2, 3, 4, and 5.

TABLE 4

*Effect of  $\text{CaCl}_2$  on phosphorus solubility and pH*

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

CaCl <sub>2</sub> , Ca EQUIVA- LENT TO TONS CaCO <sub>3</sub> INDICATED PER ACRE	BASE SOIL WAS TREATED WITH															
	H		Fe		Al		None		Mg		Ca		NH <sub>4</sub>		K	
	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH	P <sub>2</sub> O <sub>5</sub>	pH
0	0 18	3 19	0 18	3 56	0 15	3 63	0 21	4 70	0 18	5 05	0 20	5 00	0 55	5 81	0 46	5 75
½	0 18	3 19	0 18	3 27	0 15	3 56	0 19	4 65	0 17	4 98	0 16	5 00	0 40	5 75	0 45	5 65
1	0 17	3 15	0 17	3 27	0 14	3 56	0 19	4 59	0 15	4 98	0 16	5 13	0 22	5 06	0 40	5 58
2	0 17	3 01	0 19	3 25	0 14	3 55	0 20	4 59	0 15	4 98	0 15	5 17	0 16	4 54	0 36	5 78
5	0 16	2 92	0 17	3 20	0 14	3 55	0 20	4 54	0 14	4 82	0 15	4 97	0 15	4 40	0 28	5 32
10	0 15	2 82	0 18	2 99	0 14	3 37	0 19	4 50	0 13	4 88	0 15	4 84	0 14	4 22	0 24	5 19

TABLE 5

*Effect of KCl on phosphorus solubility*

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

KCl, POUNDS PER ACRE	BASE SOIL WAS TREATED WITH							
	H	Fe	Al	None	Mg	Ca	NH <sub>4</sub>	K
0	0 20	0 21	0 14	0 27	0 19	0 19	0 55	0 49
100	0 18	0 19	0 14	0 24	0 19	0 18	0 55	0 46
250	0 18	0 17	0 14	0 24	0 16	0 19	0 55	0 41
500	0 17	0 18	0 14	0 23	0 20	0 18	0 55	0 40
750	0 20	0 16	0 13	0 21	0 19	0 17	0 56	0 43
1,000	0 20	0 21	0 14	0 23	0 20	0 18	0 56	0 37

## DISCUSSION

An examination of these data shows conclusively that the cation absorbed by the soil complex greatly affects the solubility of phosphorus. The first lines in tables 2, 3, 4, and 5 show that in using the untreated soil as a standard, the replacement of the absorbed bases by Al, Fe, H, Mg, and Ca decreases the soluble phosphorus, and that replacement of the bases with NH<sub>4</sub> or K increases the soluble phosphorus. Apparently, under the conditions governing our work, absorbed aluminum precipitates most completely the soil phosphorus, followed by magnesium and iron and then hydrogen and calcium. It is probable that the precipitation caused by hydrogen is due to iron or aluminum which has been dissolved by the high degree of acidity. It may be noted that

with various bases held by the absorbing complex of the soil, the soluble phosphorus remains quite constant and the pH varies considerably. In particular note the H, Fe, Mg, and Ca soils which, unfertilized, have approximately 0.20 p.p.m. of soluble phosphorus, and whose pH values vary from about 3.00 to about 5.00. The results obtained as to the effect of absorbed base agree with the findings of Comber (4), Smirnov (11), Spurway (12), and Kappen and Bollenbeck (7).

The data in table 2 show that for soils treated with Al and Fe it is necessary to add 1,250 and 1,500 pounds of  $\text{CaH}_4(\text{PO}_4)_2$  per acre before the soluble  $\text{P}_2\text{O}_5$  in the soil is appreciably increased. That this is attributable to absorbed base rather than reaction is indicated by the H soil, which, although somewhat more acid in reaction than the Al or Fe soils, yet shows an appreciable increase in soluble phosphate with an application of 250 pounds of  $\text{CaH}_4(\text{PO}_4)_2$  per acre. It should be noted in table 2 that applications of 1,250 pounds per acre of  $\text{CaH}_4(\text{PO}_4)_2$  to the Al soils or even 2,000 pounds to the Fe soil did not result in as much soluble phosphate as was present in the unfertilized  $\text{NH}_4$  and K soils, even though these fertilizer treatments increased the phosphate content of the soils by 34 per cent and 55 per cent respectively. That the hydrogen, calcium, and magnesium soils also precipitate the phosphorus is evident from tables 2, 3, 4, and 5. In the case of these three soils, the data in table 2 show that 250 pounds per acre of  $\text{CaH}_4(\text{PO}_4)_2$  is required to increase the soluble phosphorus appreciably, and around 250 to 500 pounds per acre is required to bring it up to that of the unfertilized  $\text{NH}_4$  and K soils. An application of 100 pounds per acre, the smallest application made, greatly increased the soluble phosphate content of the  $\text{NH}_4$  and K soils.

It will be seen from the data that, excepting the hydrogen and untreated soils, the more alkaline the absorbed base makes the soil, the more soluble is the phosphorus in that soil, and the less precipitating power does that soil have for added phosphorus. The power of the soils containing various exchangeable bases to precipitate  $\text{P}_2\text{O}_5$  agrees with the work of Nemec (8) and Nemec and Gracinin (9).

In table 3 are presented the data resulting from the studies of the effect of applications of calcium carbonate to the soils. In the cases of the H, Ca,  $\text{NH}_4$ , and K soils the  $\text{CaCO}_3$  decreases the solubility of the phosphorus. Theoretically this would be caused by the substituting of a more insoluble calcium phosphate for a more soluble calcium phosphate, or the H,  $\text{NH}_4$ , or K phosphates. The substitution of a more insoluble calcium phosphate might be replacing a dibasic salt by a tribasic or a dibasic salt for a monobasic. In the case of the Fe, Al, Mg, and untreated soils the applications of  $\text{CaCO}_3$  have increased the solubility of the soil phosphorus. This increased solubility might be caused by the precipitating of the Al and Fe ions by the basic reaction, and the substituting of the more soluble calcium phosphates for the less soluble phosphates of iron or aluminum. In every case the addition of calcium carbonate has made the soil more basic. This effect of  $\text{CaCO}_3$  increasing the

solubility of phosphorus agrees quite well with the work Askinazi and Yarusov (2), Wityn (15), Antanov (1), and Demolon and Barbier (5).

That the increased solubility of  $P_2O_5$  in the case of Fe, Al, Mg, and untreated soils is due to the change in reaction rather than to the presence of the calcium, is made evident by comparing the data in tables 3 and 4. In table 4 the results of the application of calcium chloride instead of calcium carbonate are presented. By this treatment the calcium was applied in a vastly more soluble form, as a salt that would have a very small effect on the soil reaction. The applications of  $CaCl_2$  contained calcium equivalent to the calcium added in the carbonate form. In no case where the calcium chloride was added did an increase in phosphorus solubility result. With the H, Fe, Al, untreated, Mg, and Ca soils  $CaCl_2$  had little or no effect on phosphorus solubility, while in the case of the  $NH_4$  and K soils  $CaCl_2$  materially decreased the solubility. In all cases application of  $CaCl_2$  rendered the reaction somewhat more acid. From the data in tables 3 and 4 it appears that applications of calcium are either inactive or tend to precipitate the phosphorus in a soil. If, however, the calcium is applied in a form that increases the alkalinity, the phosphorus solubility might be increased, probably as a result of secondary reactions.

In view of the fact that theoretically potassium fertilization might relieve phosphorus deficiencies by increasing the solubility of the soil phosphorus due to a non-common ion, the effect of applications of potassium chloride has been investigated with the eight soils. The results reported in table 5 show little or no effect on phosphorus solubility with applications of KCl up to 1,000 pounds per acre. The physico-chemical law that the addition of an unlike ion will increase the solubility of the ions already present would lead one to expect an increased phosphorus solubility. This result was not found when working with Cherokee loam, regardless of the base contained in the soil.

Attention should be called to the data in table 3. Here it is seen that with heavy applications of lime, more soluble  $P_2O_5$  appears in the iron and aluminum soils than in the hydrogen soil. These data have been re-checked and a further investigation of these solubility effects is in progress.

#### SUMMARY AND CONCLUSIONS

The absorbed bases in separate portions of Cherokee loam were replaced with H, Fe, Al, Ca, Mg, K, and  $NH_4$ , and an untreated portion was also retained. The eight soils thus prepared were treated with  $CaH_4(PO_4)_2$ ,  $CaCO_3$ ,  $CaCl_2$ , and KCl in varying amounts, and the water-soluble phosphorus was determined.

The soluble phosphorus of soil is increased by replacing the absorbed bases by K or  $NH_4$ .

When phosphorus is applied as  $CaH_4(PO_4)_2$  the Fe and Al soils precipitate large amounts of phosphorus; the H, Mg, Ca soils, intermediate amounts; and the K and  $NH_4$  soils, small amounts.

In acid soils with similar pH values, less phosphorus will be precipitated on the application of phosphatic fertilizers when the acidity is caused by absorbed hydrogen rather than by the hydrolysis.



$\text{CaCO}_3$  applications increase the soluble phosphorus in the Fe, Al, and Mg soils, but decrease it in the H, Ca,  $\text{NH}_4$ , and K soils.

Phosphorus solubility is increased by lime on soils made acid by absorbed Fe or Al, but is decreased on soils made acid by absorbed H.

The increased phosphorus solubility resulting from lime is attributed to the reaction rather than to the calcium, since applications of  $\text{CaCl}_2$  failed to increase the solubility.

The solubility of the phosphorus in a soil is not affected by the application of KCl, regardless of the base held by the soil.

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# A SIMPLE AND RAPID METHOD FOR MEASURING THE STICKINESS OF SOILS<sup>1</sup>

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In previous communications (2, 4) studies have been reported on the relationship that exists between certain physical properties of soils, such as moisture equivalent, unfree water, heat of wetting, flowing point, upper plastic limit, and crumbling point, and their clay and colloidal content as determined by the hydrometer method (3) of mechanical analysis.

Stickiness is the next physical property of soils whose relationship to clay and colloidal content it was undertaken to investigate. This property is prominent in nearly all of the fine textured soils and has a great bearing on their tilth, resistance to crushing, water percolation, and handling. It was found, however, that there was no satisfactory method for measuring quantitatively the degree of stickiness in soils and hence it was necessary to devise a method. After some experimentation a simple and rapid method was devised which gave a satisfactory quantitative measurement of the maximum stickiness in different soils. It is the object of this paper to present this new method and the experimental results obtained with it.

## METHOD AND PROCEDURE

The principle of the method consists in pressing a small metal disk against the surface of a thoroughly puddled and kneaded soil, and then pulling this metal disk vertically upward by means of a spring balance upon which the force of pull is directly recorded. The moisture content of the soil is gradually varied until the maximum pull is obtained.

The following pieces of apparatus are required for making the determination: a metal disk, a recording spring balance, two spatulas, a base, a pan, a pipette, and a balance for weighing soil.

The metal disk is made of sheet copper 2 inches in diameter and  $\frac{1}{16}$  inch in thickness and has a small ring exactly in the center of the upper surface. The total area of the disk is 3.14 square inches. The metal is of sufficient thickness and strength not to bend.

The spring balance is vertical and reads to 30 pounds in  $\frac{1}{4}$ -pound divisions and 15 kilos in 100-gm. divisions. It has a sliding recorder which remains at

<sup>1</sup> Authorized as Journal Article No. 93 (U. S.) from the Michigan Agricultural Experiment Station.

the maximum pull reached after the spring is released and the indicator goes back to zero. This spring balance together with the metal disk is shown in plate 1.

Two spatulas are required, one with a thin blade that will bend easily and one with a stiff blade that will not bend. The flexible spatula is used mainly in gathering the soil into a mass or mound, and the non-flexible blade is employed principally in puddling and working the soils to reduce them to the particle state, and to develop their stickiness.

For a base it was found most desirable and convenient to use an iron block 5 inches square, 4 inches deep, with a rather smooth surface and weighing about 35 pounds. This kind of base makes it much easier to work the soils and to press the metal disk down on them.

The pan is used to mix the dry soils with water at the beginning so that none will be lost. After the soils are moistened they are transferred to the iron block where all subsequent mixing and working are done.

The procedure adopted consists in weighing out 30 gm. of air-dry soil which has passed through a 2-mm. sieve, spreading it in a thin layer in the pan, and adding a sufficient quantity of distilled water from a graduated pipette to make it just moist enough so it can be kneaded. After being thoroughly mixed the soil is removed to the iron block and worked vigorously with the spatula to obtain as complete dispersion as possible. Various movements can be employed to knead and disperse the soil, but at the lowest moisture content the one that seems to be the easiest and most efficient is to slice the soil into thin layers by a shearing stroke of the spatula and press it or rub it on the iron surface. The soil is again gathered into a mass or mound and the process of kneading and working is repeated many times until the soil is thoroughly dispersed. Finally, the whole soil mass is gathered into a mound by the spatulas and the metal disk is pressed on it with considerable force until there is an intimate and complete contact between the surface of the disk and the soil. The spring balance is then hooked through the ring on the metal disk and a gradual, vertical pull exerted, the balance being held firmly between the hands, until the disk breaks away from the soil. The pull required to break the contact is recorded. The soil is gathered into a mound again and the determination repeated several times in order to obtain a series of readings from which to get an average. If the testing is carefully done the determinations will agree closely. In case they do not agree the soil is insufficiently dispersed or sufficient care has not been exercised to obtain complete contact between the disk and soil. To the same soil sample is then added 1 cc. more water and the process of mixing, kneading, gathering it into a mound, and determining the pull required to break the disk away from the soil is repeated. The whole process is repeated until a maximum pull is reached and passed. It usually takes about an hour to determine the maximum stickiness of most soils.

For a satisfactory application of this method the following essential points

must be strictly observed: (a) The soil should be as completely dispersed as possible in order to develop its potential maximum stickiness. This end is undoubtedly accomplished by using the same soil sample in all the different determinations, and by starting with a low moisture content at which a soil can be more effectively dispersed than at a high moisture content where the compound particles slide easily over one another. (b) The metal dish should be pressed with sufficient force, which is very considerable at the lower soil moisture contents, to produce a very intimate contact between its surface and the soil. On the other hand, extreme care must be taken not to press the disk down so hard that it displaces most of the soil, and allows its surface to come very nearly in contact with the surface of the base. When this is done another external factor comes into play which tends to vitiate the results. It is only at the higher moisture contents when the soil is very soft, however, that it is easily displaced. (c) The surface of the disk should be cleaned with a wet cloth and wiped dry every time the disk is used, especially at the lower moisture contents. If soil dries on the disk, especially in the form of dust, it seems to prevent the moist soil sticking to the disk. In this connection it might be said that fingers are not especially good objects with which to test stickiness. (d) The disk should be pressed down as level as possible and should be pulled up straight and gradually, and not with a jerk. (e) It is advisable to gather the soil into a high mound and the press then disk down on it until the surface of the soil equals that of the disk.

When the disk is pulled up, the break almost always takes place in the soil mass and not between the surface of the disk and the soil. Soils at very low moisture content, and soils possessing little stickiness may be exceptions to this rule.

Since the same soil sample is employed for all determinations, the moisture content at each measurement can only be approximately calculated from the amount of water added. This, however, is of no serious importance since a knowledge of the exact water content is not essential.

Although the method cannot be considered to be highly accurate and sometimes tends to give somewhat erratic results, yet by following the technique as developed and by observing the precautions mentioned, fairly satisfactory results are obtained.

The term "stickiness" is employed here to include all the forces that the conventional terms "adhesion" and "cohesion" are supposed to represent. Critical examination tends to show that there is no fundamental difference (1) between adhesion and cohesion and especially in soils which are not a homogeneous mass but rather a heterogeneous mass with water playing a leading rôle in the development and manifestation of these forces.

#### EXPERIMENTAL RESULTS

In table 1 are presented the experimental results on the stickiness of a large number of representative types of soil. The data are expressed in pounds

TABLE 1  
Stickiness of Different Soils in Pounds Pull Per Square Inch at Various Water Contents; with Special Emphasis on the Maximum Stickiness

SOILS	STICKINESS IN POUNDS PULL PER SQUARE INCH AT MOISTURE PERCENTAGES OF																			
	20.0 %	23.3 %	26.6 %	30.0 %	33.3 %	37.0 %	40.0 %	43.3 %	46.6 %	50.0 %	53.3 %	56.6 %	60.0 %	63.3 %	66.6 %	70.0 %	73.3 %	76.6 %	80.0 %	83.5 %
1. Muck.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Plainfield sand, surface.....	0.08	0.09	0.07																	
3. Dickson sandy loam surface.....	1.0	1.2	1.0	0.80																
4. Clarion loam, subsoil.....	0.692	0.89	0.40																	
5. Clarion loam, surface.....			2.39	3.18	3.34	2.86	2.07													
6. Lindy silt loam, surface.....	1.41	2.89	2.98	2.82	2.20	1.70														
7. Clinton silt loam, surface.....			1.80	2.67	2.20	1.25														
8. Putman silt loam, surface.....																				
9. Grundy silt loam, surface.....			1.91	2.55	3.19	2.86	2.55	1.59												
10. Edena silt loam, 0-9 inches.....					2.67	3.30	3.92	3.77	3.19	2.83										
11. Antioch clay loam, 40-60 inches.....	3.49	4.08	4.73	5.34	5.18	4.24	3.30	2.51												
12. Almont adobe, 0-10 inches.....			4.30	5.10	5.41	5.57	5.73	4.46	3.02	3.82	3.82	3.19	2.71	2.55	2.39	2.07				
13. Susquehanna clay, 16-20 inches.....			2.55	3.50	4.30	4.01	4.01	4.01	3.82	3.82										
14. Davidson loam, 18-30 inches.....											2.83	2.98	2.83	2.83	2.83	2.51	2.51			
15. Decatur clay, 6-12 inches.....			4.46	4.62	4.81	4.30	3.82	3.19												
16. Ontonagon clay, C horizon.....			5.18	6.28	6.91	6.44	5.50	4.55												
17. Haldemand clay, subsoil.....			4.50	5.49	5.81	5.65	4.08	3.49												
18. Capay clay, adobe.....			5.57	6.85	7.49	7.96	7.80	6.85	6.68	6.37	5.73	5.10	3.53							
19. Lake Charles clay, surface.....			3.96	5.10	5.73	5.48	3.02													
20. Fargo silty clay, subsoil.....				5.78	6.30	6.90	7.50	7.45	7.40	7.20	7.00	6.40	6.0	5.1						
21. Fargo clay, 6-16 inches.....			3.19	3.49	5.34	7.54	7.54	7.69	7.85	7.69	7.54	6.12	5.02	4.40						
22. Fulton clay, subsoil.....			5.43	6.30	7.0	7.10	7.0	6.5	6.0											
23. Fargo silty clay, 3-8 inches.....																				
24. Black clay, adobe.....																				
25. McKenzie clay, Ba.....				5.35	5.60	6.80	7.20	6.90	6.80	5.60	5.00	4.33								
26. Soils 8 and 25 mixed half and half.....				5.88	7.90	9.88	12.8	45.8	92.8	99.9	30.9	30.9	05.9	01.8	70.8	0	7.60	6.30	5.50	
27. Mixture $\frac{1}{2}$ soil 25 and $\frac{1}{2}$ soil 8.....				5.75	6.80	7.50	7.07	90.7	30.6	50.5	80.4	90								
28. Soils 8 and 16 mixed half and half.....				4.40	6.0	6.20	5.80	5.05	4.20	3.60										
29. Soils 2 and 25 mixed half and half.....				3.98	5.20	4.90	3.82	90.2	58											
30. Mixture $\frac{1}{2}$ soil 2 and $\frac{1}{2}$ soil 25.....			8.84	9.00	12.8	50.8	10.7	17.6	50.5	57.4	50									
31. Soils 1 and 25 mixed half and half.....	6.98	7.94	7.27	7.01	6.05	4.46	3.18													
32. Mixture $\frac{1}{2}$ soil 1 and $\frac{1}{2}$ soil 25.....								2.90	4.80	5.10	5.50	5.55	10.4	90.4	01.3	60				
								5.09	6.70	6.98	7.40	7.80	7.40	6.80	5.90	5.01	4.60			

pull per square each. Each measurement is the average of five or more trials. The data in table 1 show that the property of stickiness varies greatly both in different soils and within the same soil at different moisture contents. In the muck, sands, and sandy loams tested there is no measurable degree of stickiness at any moisture content, but in the loams and especially in the clays there is comparatively a very high degree of stickiness over a wide range of moisture content. Stickiness tends to increase more or less with the moisture content until a maximum is reached and then it decreases. Every soil appears to have a definite maximum degree of stickiness, the magnitude of which ranges from 0.0 pounds pull per square inch in muck to 0.09 pounds in Plainfield sand; 3.10 pounds in Putman silt loam; 5.73 pounds in Lake Charles clay; 7.50 pounds in Fargo silty clay; and 9.30 pounds in McKenzie clay. The last figure, which is the largest obtained in the soils investigated, represents really a tremendous amount of stickiness.

It will be observed that the maximum stickiness remains practically the same for a considerable range of moisture in nearly all of the heavy soils but especially McKenzie clay, Davidson loam, and Fargo clay loam. This is probably due to the fact that as the soils are being dispersed their consistency tends to remain almost the same for a considerable range of moisture content until they are completely dispersed. This explanation finds support in the previous work on the flowing point of soils (4). When the soils are not completely dispersed an apparent maximum stickiness is reached more abruptly but the result may not be the maximum stickiness which it is possible to develop in the soil.

The results for soils 14, 29, 30, 31 and 32 deserve special attention. Soil 14, which is Davidson loam and a laterite, contains 71.2 per cent clay and yet has very little stickiness. The same is true of Nipe clay, which is a more typical laterite than the Davidson loam. Apparently lateritic soils possess very little stickiness. Soil 29 is a mixture of one part of Plainfield sand and one part of McKenzie clay, and soil 30 contains two parts of Plainfield sand and one part of McKenzie clay, yet these mixtures manifest as high stickiness as the McKenzie clay alone. In other words, diluting this extremely sticky soil with two parts of sand to one part of clay does not perceptibly decrease its maximum degree of stickiness. On the other hand, diluting this same McKenzie clay with muck causes its stickiness to be markedly reduced as shown by the result for soils 31 and 32, which contain one part of muck to one part of clay, and one part of muck to two parts of clay, respectively. Apparently muck has a great influence in reducing stickiness whereas sand has practically none, at least, in the proportions tried.

#### RELATIONSHIP BETWEEN MAXIMUM STICKINESS AND THE CLAY CONTENT OF SOILS

After the maximum degree of stickiness was determined in the different soils, it was decided to ascertain what relationship this property bears to the clay (0.005 — 0 mm.) and clay (0.002 — 0 mm.) as determined by the hydrometer method (3). This relationship is revealed in table 2.

From the last two columns of table 2, which show the ratio between maximum stickiness and clay and fine clay, it is readily seen that out of 22 soils examined, 20 show fairly close relationship between their maximum degree of stickiness and their clay and fine clay content. The two exceptions are Davidson loam, which is a laterite, and Haldemand clay subsoil.

The results of table 2 indicate that, on the whole, stickiness is controlled mainly by the clay and colloidal content, although in certain cases some other factor, possibly composition, is also a determining factor. In lateritic soils there is distinctly no relationship between their stickiness and clay content.

TABLE 2  
*Comparison Between Maximum Stickiness and Clay Content of Soils*

SOILS	MAXIMUM STICKI- NESS	CLAY	FINE CLAY	RATIO CLAY STICKINESS	RATIO FINE CLAY STICKINESS
	<i>pounds</i>	<i>per cent</i>	<i>per cent</i>	<i>ratio</i>	
Dickinson sandy loam, surface . . .	1 2	13 5	12 5	11 3	10 4
Clarion loam, surface. . . . .	3 34	24 3	21 8	7.28	6 53
Lindy silt loam, surface. . . . .	2.89	25 7	20 6	8 90	7 13
Clinton silt loam, surface. . . . .	2 67	29 2	23 4	10 9	8 76
Putman silt loam, surface. . . . .	3 10	24.2	20 5	7.81	6 61
Grundy silt loam, surface. . . . .	3.19	33.2	29 2	10 4	9 16
Edena silt loam, 0-9 inches. . . . .	3 92	36.1	30 7	9.21	7 83
Antioch clay loam, 40-60 inches . . .	5 34	34 8	32 4	6 52	6 06
Almont adobe, 0-10 inches . . . . .	5 73	51 8	45 9	9 04	8 01
Susquehanna clay, 16-20 inches. . . .	4 30	41 0	39.5	9 54	9 18
Davidson loam, 18-30 inches . . . . .	2 98	71 2	69.1	23 9	23 2
Decatur clay, 6-12 inches. . . . .	4 81	42 3	40 7	8.80	8 46
Ontonagon clay, C. . . . .	6.91	79 8	72 7	11 6	10 50
Haldemand clay, subsoil. . . . .	5 81	85 6	77 4	14 7	13 3
Capay clay, adobe. . . . .	7.96	63 2	56 9	7 94	7.15
Lake Charles clay, surface. . . . .	5 73	42 4	38 9	7.40	6 79
Fargo silty clay, subsoil. . . . .	7 50	69.7	64 6	9.29	8 63
Fargo clay, 6-16 inches. . . . .	7.85	60.4	55 1	7 69	7 02
Fargo silty clay, 3-8 inches. . . . .	7.80	70 2	65 8	9 00	8 45
Fulton clay, subsoil. . . . .	7.10	68.0	66.5	9.58	9 36
Black clay, adobe. . . . .	7 20	55 9	52.7	7.76	7.32
McKenzie clay, B <sub>2</sub> . . . . .	9 30	76 1	71 9	8 18	7.73

High organic matter content especially in undecomposed forms reduces markedly the stickiness. Carbonates do not seem to exert much effect, judging from the results from Ontonagon clay, C horizon, and Fulton clay subsoil, both of which contain a very high content of carbonates but in the colloidal size.

#### RELATIONSHIP BETWEEN MAXIMUM STICKINESS AND UPPER PLASTIC LIMIT

In order to determine what relationship exists between the upper plastic limit of soils and their maximum stickiness, a comparison was made of the two properties and is presented in table 3.

From the results in table 3 and especially those in the last column, it is apparent that for the majority of the soils investigated there is some, though not a very close, relationship between their maximum stickiness and upper plastic limit. In the case of certain soils, particularly the lateritic Davidson loam, no relation appears to exist between these two properties.

TABLE 3  
*Comparison Between Maximum Stickiness and Upper Plastic Limit of Soils*

SOILS	UPPER PLASTIC LIMIT	MAXIMUM STICKINESS	RATIO U.P.L. M.S.
	<i>per cent</i>	<i>pounds</i>	
Lindy silt loam, surface . . . . .	27.2	2.89	9.41
Clinton silt loam, surface . . . . .	29.9	2.67	11.30
Putman silt loam, surface . . . . .	37.4	3.10	12.00
Clarion loam, surface . . . . .	31.5	3.34	9.43
Edena silt loam, 0-9 inches . . . . .	39.5	5.34	7.40
Almont adobe, 0-10 inches . . . . .	39.1	5.73	6.82
Susquehanna clay, 16-20 inches . . . . .	46.0	4.30	10.70
Decatur clay, 6-12 inches . . . . .	32.0	4.81	6.65
Ontonagon clay, C. . . . .	36.3	6.91	5.25
Lake Charles clay, surface . . . . .	39.6	5.73	6.91
Davidson loam, 18-30 inches . . . . .	59.8	2.98	20.10
Haldemand clay, subsoil . . . . .	41.3	5.81	7.11
Capay clay, adobe . . . . .	41.80	7.96	5.25
Fulton clay, subsoil . . . . .	39.8	7.10	5.60
McKenzie clay, B <sub>2</sub> . . . . .	71.1	9.30	7.64

#### SUMMARY

A simple, rapid, and fairly accurate method has been developed to measure quantitatively the maximum stickiness in soils.

The results obtained show that this maximum degree of stickiness measured in pounds per square inch required to pull a metal disk from the soil, varies from 0.0 pounds in muck and sand to about 10 pounds in some of the clays.

There is a fairly close relationship between the maximum stickiness and clay content in most soils, but in some soils this relationship is very slight.

There is also some, though not a close, relationship between the maximum stickiness and the upper plastic limit in most soils, but there are some very distinct exceptions.

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PLATE 1

SPRING BALANCE AND DISK FOR MEASURING THE STICKINESS OF SOILS





## **Professor K. K. Gedroiz**

The staff of the department of soil science of the New Jersey Agricultural Experiment Station, and of the College of Agriculture of Rutgers University have learned with keen sorrow and regret of the death of Prof. K. K. Gedroiz, and, with their colleagues at other institutions and other countries, recognize his passing as a most serious loss to science and agriculture. The young but rapidly growing science of the soil has lost in Professor Gedroiz an outstanding scholar whose contributions to our knowledge of the base exchange capacity and the colloidal properties of the soil have revolutionized our ideas concerning this important branch of soil science. As president of the Second International Soil Science Congress that convened in Russia in 1930, he contributed materially by his great reputation toward making the congress a success, even though ill health prevented him from taking a part in the deliberations of the congress. Russia has given many great men to soil science, and the name of Gedroiz will be remembered with those of Dokutschaiev, Sibirtzev, Kossowitch, Glinka, and many others, as having laid the foundation of a new science, which is at the very base of agriculture. The director and the members of the staff of the New Jersey Agricultural Experiment Station and of the College of Agriculture of Rutgers University wish to convey to the Academy of Sciences of the U. S. S. R. and to their colleagues in the Union and in other countries this message of sympathy and profound regret.



## **Academician Konstantin Kaetonobitch Gedroiz**

**1872-1932**

Within the last five years, it has become the sad task of the writer to record the deaths of Russian scientific men in the field of soil science and plant nutrition. One after another, within this short period of time there passed away, first Glinka, then Omeliansky, Neustruev, Kostytchev, and now Gedroiz, who died from arteriosclerosis on October 5. All were brilliant investigators. All have made outstanding contributions to the new but rapidly growing science of the soil: Glinka and Neustruev, in the field of genesis and classification, Omeliansky and Kostytchev in the field of microbiology, and Gedroiz in the field of chemistry. None was more original and none has contributed more toward revolutionizing the prevailing ideas in soil science than Gedroiz.

Gedroiz was born in 1872 in Bender, Government of Bessarabia, the son of a physician. He was the next to the last child of a large family. Gedroiz can be considered truly international in nature, both by birth and education. His father was the son of a Lithuanian (father) and a Pole (mother); his mother was the daughter of a Hungarian (father) and a German (mother). His childhood was spent in peasant surroundings in the southern part of Bessarabia (now Roumania), amidst Bulgarians, Turks, Roumanians, Greeks, and Russian Raskolniks. He received his preliminary training at a Real school in Kiev and his higher education at the Forestry Institute, in St. Petersburg, from which he graduated in 1897. As a student, he became interested in science, utilizing the opportunity which was presented at the soil science laboratory of P. to L. Kossowitch of the Forestry Institute. His first investigation dealt with the solubility of the phosphoric acid of the Russian low grade phosphates in various solvents. The data obtained were utilized by Kossowitch for his work on the Russian low grade phosphates (Volume II of the Works of the Agricultural Chemistry Laboratory of the Agricultural Institute in St. Petersburg).

On graduation from the Forestry Institute, Gedroiz was invited by Kossowitch to assist him in the organization of the Agricultural Chemistry Laboratory of the Agricultural Institute. From that time until 1915 he served this institute, as scientific collaborator in the laboratory and in charge of the greenhouse experiments. He continued, simultaneously, his training at the University of St. Petersburg, in the Faculty of Natural Science and Mathematics and received his diploma in 1903. Beginning with 1900 he took an active part in the publication of the *Journal of Experimental Agronomy* under the editorship of Kossowitch, first as collaborator and assistant editor, and, when Kossowitch died (1915), as editor.

In 1908, Gedroiz undertook a series of scientific investigations at the Bureau

of Agriculture and Soil Science of the Scientific Commission of the Agricultural Institute. He continued his connection with this institute, which frequently changed its name, until 1928. His first investigations were the now famous studies of the soil colloidal complexes and the absorbing capacity of the soil. These resulted in a series of brilliant papers, which, unfortunately, as a result of the World War and the Russian revolution, remained unknown to the outside world until 1923, when a mimeographed edition of an English translation of a selected group of these papers was made available in America. In 1926, a summary of these papers was presented at the Conference of the Second Commission of the International Society of Soil Science in Groningen by Dr. Page. When the writer visited, in 1924, the famous German soil chemist Ramann, he found him deeply immersed in the study of Gedroiz's papers in the English translation, although Ramann himself knew some Russian.

In 1919, Gedroiz was elected by the faculty of the Leningrad Forestry Institute as professor of soil science. In 1927, he was elected correspondent of the Academy of Sciences of U. S. S. R. and was given the Lenin prize for his work. In 1929, the Academy of Sciences elected him an active member. In 1930, because of his election to active membership in the Russian Academy of Sciences, he gave up his active teaching, of which he was never very fond. In 1929, Gedroiz was elected director of the Soil Science Institute of the academy in the name of Dokutchaevev. In addition to these positions, he also had charge, during this period of time, of the soil chemical laboratory of the Experimental Forestry Institute and of the laboratory of the Pedological Dokutchaevev Commission (1916-1918); he lectured on soil science at the Leningrad Polytechnicum (1919-1921) and in the Leningrad Agronomical Institute (1918-1922). From 1921 to 1929, he took an active part in the Nossov Experimental Station, and at the time of his death he was an active member of the Dolgoprudnoe Experimental Field of the Scientific Institute of Fertilizers. Following the death of Professor Glinka, Gedroiz was unanimously elected in 1930 as president of the Second International Congress of Soil Science.

Gedroiz's scientific contributions comprise over 100 scientific papers, published largely in the *Journal of Experimental Agronomy* and in the *Contributions of the Agricultural Chemical Laboratory of the Agricultural Institute*. In addition to these, he wrote a book on chemical analysis of the soils, which was translated into German; at the time of his death, he was in the process of preparing the third Russian edition. He also wrote a monograph on "The Absorbing Properties of the Soil," which was also translated into German.

In 1931, the *Journal of Experimental Agronomy*, of which he had been editor since 1915, ceased publication. (Only a few issues had appeared at irregular intervals since 1926.) Its place was taken by a new journal, *Chemization of Socialistic Agriculture*, of which he was made one of the editors and an important contributor.

The investigations of Gedroiz deal primarily with the physical, physico-chemical, and chemical properties of the soil. The origin of the soil and its

degradation; the formation of saline, alkaline, and acid soils; and classification of soils on the basis of their absorptive power, all received careful consideration and his contributions to this field are most outstanding. On the basis of these investigations, he has drawn a clear picture of the soil, especially the soil absorbing complex.

Personally, professor Gedroiz was very modest and unassuming. The civil war in Russia, following the revolution, has left a marked impress upon his family. With a brother killed in the revolution, one son killed in the war, another son maimed for life, the only daughter just having passed through an epidemic of typhus, he was still able to continue his work almost uninterruptedly except for the physical inconveniences and lack of sufficient supplies. When the writer of these lines was entertained at the home of Professor Gedroiz at the Forestry Institute in Leningrad in 1924, the writer expressed his great surprise at Gedroiz's ability to carry on under these circumstances (some of his best work appeared during that period). Professor Gedroiz replied with a smile: "We must adjust our lives, the scientific work must go on, at any cost." His wife was his constant companion and shared with him cheerfully all his difficulties.

During 1921 and 1922, Gedroiz carried out his studies under the most difficult and trying circumstances. Frequently the most essential pieces of apparatus and supplies were completely lacking and could not be procured at any price. Without complaint, however, he carried out his work.

His letters were always sincere and full of enthusiasm. The following few extracts from an extensive correspondence which lasted uninterruptedly for ten years, will serve better to illustrate the nature of the man and his devotion to his work and to his family than any amount of description.

June 17, 1923

"Dear Colleague:

"I am in receipt of your kind letter. Will be very glad if you will send me a copy of your translation of my papers; I will look it over, possibly change something here and there and supplement it further in accordance with my new experimental data. At present, the status of printing is at a very slow pace with us; my last papers, dealing with solonchaks, are awaiting their turn at the printers. As soon as they appear, I will send them to you.

"We are here in great need of American scientific literature. The *Journal of Experimental Agronomy* (*Zhurnal Obitnoi Agronomii*), of which I am editor, has ceased to receive, since 1918, American scientific literature, including the publications of the U. S. Department of Agriculture. We have only the first 4 volumes of *Soil Science*. The Journal has no money to subscribe for books and other journals. I can only offer an exchange arrangement; we have available the complete files of the Journal for 1909-1920, while the issue for 1921-1922 is being published now. I would be very thankful to you if you could help the *Journal of Experimental Agronomy* to receive the American journals



in the field of agriculture, soil science, plant nutrition, as well as the publications of various experiment stations. You could thus render a great service to Russian agronomists."

August 6, 1923

"I am in receipt of your letter of 12/VII and the translations of my three papers. I am very much indebted to you for your trouble concerning American scientific literature. I have glanced through the translations. I am not as good an English scholar as to be able to be a good judge, but I liked the translation; it seems to me quite accurate and one can read it readily. I have begun a number of investigations, but have not brought them as yet to a state where the results can be published. The conditions for scientific work are very difficult here: for example, since 1919 there has been no gas in Leningrad. We have to use kerosene or denatured alcohol, which is very expensive and inconvenient. One has to do everything himself, including dish washing. Conditions as they are force one to give up scientific work altogether and do something else. But by force of habit, established during a period of nearly 25 years, one does his work and cannot leave the laboratory in spite of the fact that it ruins one's health, due to the difficult material conditions and the most unfavorable conditions for carrying out one's work."

February 9, 1924

"In regard to my last papers (sent in original copy, in an unpublished form), I believe it will be best to have them mimeographed (do not forget, please, to send me a copy of each). They are already set in type for the *Journal of Experimental Agronomy*, but it is quite impossible to say when they will be published, due to a lack of paper and money at the present time; possibly soon, and possibly only in a year from now or later. . . . It is better to have them mimeographed. I am now completing my last paper, "Soil absorbing complex and the absorbed soil cations as a basis for a genetic soil classification." Possibly it will appear in print here, and possibly not! If not, I will then ask you to see whether it cannot be placed in *Soil Science*. The paper is of general interest. We may meet in Rome, if not—I hope to see you in our home in Leningrad."

March 9, 1924

"I am at present much overburdened with teaching duties at the Forestry Institute. There are a great many students who are striving to obtain knowledge of the soil; the conditions are not sufficiently favorable, however, to satisfy these growing interests. One has to spend, therefore, much time in preparation. I shall hardly get to Rome. The chief difficulty lies in the fact that my

son is very sick, for nearly a year now; the nature of the disease is such that it is almost impossible for me to be away. I do hope that we will meet in Leningrad . . . .”

January 6, 1925

“ . . . . My work does not progress very well. One has to devote altogether too much time to teaching students, and due to their large numbers and very limited facilities and means available for teaching, it is not such an easy problem, especially since I am not primarily a teacher by nature. However, without a professorship, it is impossible to live and devote oneself only to scientific work. Since materially our condition is also not of the very best, the purely scientific investigations are very much hampered. The monograph concerning the solonchets is far from progressing, due to above circumstances. However, my new system of soil classification is already published, and will arrive within a few days from the printers. It has an extensive summary in German, but I would like very much to see it published in English. It forms in a way a completed philosophy of all my previous work. Perhaps Dr. Scofield will be interested in having it mimeographed, if it cannot be printed . . . .”

July 27, 1925

“Your letter of the 29/VI did not reach me in Leningrad but was forwarded to me to the Nossov Experimental Station (Government of Tchernigov) where I am spending the time now partly for the purpose of resting and partly for continuing my investigations, especially in order to keep in touch with the soil. I hope very much that I shall be able to come to America in 1927, to see and become acquainted with American life and work. I am planning to begin this winter to study English, in order to understand the language and make myself understood.”

April 29, 1926

“ . . . . Concerning my life, there is nothing to boast of. My son is in a very critical condition; it seems that his case is hopeless. My daughter is not well so that it will be necessary to go south during the summer, probably to Crimea. During a period of nearly five years, when they were both at an age of transition, dangerous for their health, they experienced want which is showing now. Even now, unfortunately, the living conditions are not sufficiently favorable, so that one can hardly expect to catch up with the former losses.

“I believe, however, that during the summer, when and if I have had a chance to rest up a little, I shall prepare a complete paper on the origin of alkali soils and will send it to you for *Soil Science* . . . .”

From a later letter:

"I began to apply for the necessary permission to come to your country for a visit; I would very much like to see your country and visit your institutions. I am only somewhat perplexed by the language difficulties. It is not very easy to learn to speak and carry on a conversation. I have always experienced language difficulties, in spite of the fact that I studied foreign languages and visited the countries of Germany and France. I learn to read very easily and quickly, but to speak is another matter.

"My investigations on the physical properties of the soil are being continued. The results obtained are highly interesting; unfortunately, the time available for this work is very limited, and I do not believe that I will be able to complete this work in time to be able to present it at the meetings of the commission in Groningen. I believe that I will be able to explain definitely this type of soil absorption, as I have done for the exchange capacity of absorption (physico-chemical). I am also beginning to outline an investigation of the mechanical absorptive power of soils (retention by soils of solid particles) . . . ."

November 18, 1926

" . . . . I am studying the English language; the progress is not as rapid as I was hoping; the time that I am able to give to it is very limited. I hope, however, that I will soon be able to write to you in English. I am afraid, however, that I will not master the pronunciation; it seems though that English comes to me much easier than German and French.

"I am waiting impatiently for the end of December when I shall be able to leave Leningrad and go to Nossovka, even if only for a couple of weeks. Only there is it possible to do the type of scientific work which resembles, even if not fully, to some extent at least, the type of work that I have done previously. Here, however, in Leningrad, one is merely pouring from one empty barrel into another; only the nerves become exhausted, and the work does not progress very far. Constantly, one is busy with all sorts of things which have nothing to do with scientific investigations. In Nossovka things are different, even if I am able to spend there twice a year only 2-4 weeks, since I have nothing to do there with administrative work. Of course if I lived there everything there would naturally be the same as here.

"As usual, I have again to ask some favor of you. I have sent to Dr. Al. Howard of the Agricultural Research Institut at Pusa my publications. The package was accepted here but was returned from abroad; nobody knows the reason. I am sending this package to you, and would ask you to forward it to Howard. I want you to excuse me, but, as you see, we Russians are totally helpless in many respects against the outside world. I have sent a registered letter to Howard; if that comes back, I will again appeal to you. . . .

"Greetings from my wife. My daughter is improving in Crimea. I do not know, however, how the Leningrad climate will affect her. My son is in a bad condition."

May 15, 1927

" . . . I am very sorry to say that I will not be able to see you again this year, since I am not going to the congress. I am extremely tired, my family affairs are not of the pleasantest; some of the investigations begun at Nossovka have to be completed; these are some of the causes which prevent me from leaving Russia. There is also another cause which has influenced my decision: the number of candidates that can go to the congress is small, while the number of those that desire to go is very large. As in all such cases, it is difficult to make a fair division, which would remove every cause of misunderstanding. In all such instances, and in this case as well, I try to step aside.

"I have certain hopes, however, of being able to visit America in the very near future, possibly even next year. I will then be able to see the things that I am interested in and not what will be shown to me."

November 15, 1927

" . . . K. D. Glinka died. He came back from America a sick man. It seems that he died from a swelling of the lungs. All our pedologists that have been to the congress have returned completely exhausted, but they were all younger and stronger so that they soon began to improve."

Early in 1928

"I have decided not to go abroad—my health does not permit, I expect to spend the whole summer in Nossovka and partly near Kiev, but do not as yet know exactly where. If you plan to come to Leningrad during the summer, I want to offer you my apartment—it is quite empty, but is looked after by the wife of one of the workers at the Pedological Institute; you can use her services. I am living now in another building, also a part of the Institute."

Kislovodsk, December 29, 1929

"I was forced to go at the beginning of December to Kislovodsk, since I felt very badly, as a result of extreme nervous exhaustion and weakening of the heart activity. I am here together with my wife and am trying to recuperate. I am forbidden every form of mental work. It looks as if several months will be required."

March 26, 1930

"I am returning within a few days to Leningrad and will begin my investigations anew. I have improved considerably in Kislovodsk, but my heart is

still weak, due to arteriosclerosis. It is quite possible that this will prevent me from taking an active part in the soil congress. My sudden leave last fall from Leningrad prevented me from completing a series of investigations on the action of hydrogen peroxide upon the soil, and from preparing for publication a paper on the influence upon plant growth of the exchangeable cations which saturate a soil. I have completed here an article of 45 pages, in which I made clear that in acid soils, both natural and those that have been made acid by the use of artificial fertilizers, there is no exchangeable aluminum, but only adsorbed hydrogen ions. Is there a possibility of publishing it in *Soil Science*? If I do not come to the congress, I hope to have an opportunity of seeing you privately."

September 25, 1930

"I am awfully sorry that I could not see you and several other colleagues during the congress. My health has become so much worse that I was ordered to keep absolutely quiet, preferably in one place and avoid trips and visits. New impulses and any physical work cause terrific pain in my heart and prevent me from working for some time. In a peaceful state, I can continue my work. I, therefore, decided to move to Moscow where there exists an experimental field (Dolgoprudnoe) as a part of the Institute of Fertilizers. I am there in charge of the agro-chemical division. A large laboratory is being built here for that purpose. Meanwhile, I use the smaller laboratories connected with the station. I should like to have your candid opinion concerning the congress, its organization and whether the foreigners were satisfied."

December 14, 1931

"My work is progressing very slowly. Conditions in Dolgoprudnoe are still unfavorable; we already have a good greenhouse, and I have been able to utilize it in my investigations on the rôle of exchangeable Ca and Mg in plant nutrition, but the chemical facilities are still poor, since the laboratory is not completed as yet. My health is poor. I have to stay in Dolgoprudnoe and not leave it. Every trip causes terrible pain. I am now spending much time in preparing the third edition of my books, 'Chemical Soil Analysis' and 'The Absorptive Capacity of the Soil.' I would like very much to see you and talk over a great many things. . . . "

Finally from the last letter, dated September 9, 1932:

"Dear Colleague!

"How are you and where are you? Have not heard from you since last spring. I am planning to undergo a cure again. Do not know as yet where, possibly in Caucasia. Beginning last July, my health has been failing. I am sending you the last 3 issues, namely 5, 6, and 7 of our new journal (*Chemization of Socialistic Agriculture*).

"I carried through this year a large vegetation experiment, where I have shown definitely that an antagonism exists between adsorbed calcium and magnesium. The injurious effect of exchanged magnesium in the field can be neutralized by the introduction into the absorbing complex of calcium, and vice versa. Upon leaving for the south, I will write to you my new address. With best greetings from all of us,

Yours,  
K. Gedroiz"

On October 10, a telegram was received from Professor Jarilov, vice-president of the International Society of Soil Science, which reads as follows:

"Fifth October deceased President Second Congress International Society Soil Science, Member Academy of Sciences, Professor Gedroiz."

So has passed a man whose whole life was devoted to the science of the soil. The future historian of this science will give an honorary place to this brilliant and indefatigable investigator.

*List of publications of Professor Gedroiz dealing with base exchange and soil absorption*

1908. Colloidal chemistry and soil science. *Zhurnal Obitnoi Agronomii (Journal Experimental Agronomy)* 9: 272.
1911. What soils are acted upon by low grade phosphates? Soils saturated and unsaturated with bases. *Zhur. Obit. Agron.* 12: 529.
1912. Colloidal chemistry as related to soil science. I. Colloidal substances in the soil solution. Formation of sodium carbonate in the soil. Alkali soils and saline soils. *Zhur. Obit. Agron.* 13: 363.
1914. Colloidal chemistry as related to soil science. II. Rapidity of reaction exchange in the soil. The colloidal condition of the soil saturated with various bases. The indicator method of determining the colloidal content of the soil. *Zhur. Obit. Agron.* 15: 181.
1915. The action of electrolytes on clay suspensions. Communication 24 from the Bureau of Agriculture and Soil Science of the Scientific Committee of the Main Department of Land Organisation and Agriculture, Petrograd, 1915.
1916. The absorbing capacity of the soil and the zeolitic bases of the soil. *Zhur. Obit. Agron.* 17: 472.
1917. Saline soils and their improvement. *Zhur. Obit. Agron.* 18: 122.
1918. Contribution to the method of determining zeolitic bases in the soil. *Zhur. Obit. Agron.* 19: 226.
1918. Contributions to our knowledge of the absorptive capacity of soils. I. Rapidity of absorption, volume of absorption, and energy of absorption and replacement. *Zhur. Obit. Agron.* 19: 269; 20: 31.
1922. On the absorptive power of soils. Editorial Committee of the People's Commissariat of Agriculture, Petrograd, 1922.
1922. Soils unsaturated with bases. A method of determining in soils the hydrogen present in an absorbed condition. Soil requirement of lime as a neutralizing agent. *Zhur. Obit. Agron.* (1924) 22: 3.
1922. The hydrochloric acid determination of cations in soils present in an adsorbed condition. *Zhur. Obit. Agron.* 22: 53.
1922. Ultra-mechanical composition of soils and its dependence on the nature of the cations present in the soil in an absorbed condition. Liming as a measure of improving the ultra-mechanical composition of the soil. *Zhur. Obit. Agron.* 22: 29.

1925. The absorbing soil complex and the absorbed soil cations as a basis for the genetic classification of soils. Nossov Agricultural Experiment Station, Agricultural Division, Paper No. 38, Leningrad.
1926. Solodization of soils. Nossov Agricultural Experiment Station.
1926. Soil as a culture medium for the growth of agricultural crops. Soil colloids and soil alkalinity. Nossov Agricultural Experiment Station.
1926. On the soil structure and its agricultural significance. *Institute of Experimental Agronomy* (3).
1928. Alkaline soils, their origin, properties and melioration. Nossov Agricultural Experiment Station.
1930. On the question of exchangeable hydrogen and on the exchangeable aluminum in acid soils. *Pochvovedenie*. 25(5): 5.
1930. Exchangeable cations of the soil and the plant: I. Relation of plant to certain cations fully saturating the soil exchange capacity. *Soil Sci.* 32: 51.
1931. Exchangeable cations of the soil and the plant: II. Liming of soil and the relation between exchangeable calcium and magnesium in soil. *Udobrenie i urozshai*. No. 11, p. 1047.
1931. Action of hydrogen peroxide upon the soil. *Udobrenie i urozshai*. Nos. 9 and 10.
1932. The soil absorbing complex, the plant, fertilization and melioration. *Chemization of Socialistic Agriculture*, No. 1.
1932. The absorbing capacity of the soil. Third edition, corrected and improved. 202 pages. Selkolchoiz.

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## PLATE 1

PROFESSOR K. K. GEDROIZ

FIG. 1. Professor Gedroiz, just preceding the World War.

FIG. 2. Professor Gedroiz lecturing at the Nossov Experiment Station on the value of vegetation experiments.



FIG. 1







# THE DETERMINATION OF EFFECTIVE STRAINS OF RHIZOBIUM TRIFOLII DANGEARD, THE ROOT NODULE BACTERIA OF CLOVER, UNDER BACTERIOLOGICALLY CONTROLLED CONDITIONS<sup>1</sup>

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The practices of crop rotation, and of ploughing under a leguminous crop, to add a larger amount of available nitrogen to the soil, date far back in agricultural history. The fact that the nitrogen content of the legumes could be increased by inoculation of soil or seed with certain bacteria, which incite the formation of nodules on the roots of the plants and fix atmospheric nitrogen in an available form, is a comparatively recent discovery,—the first recorded experiment establishing this fact having been performed in 1887.

In 1914, it became evident that many characteristic differences were associated with and restricted to the development of any one organism from certain well-defined groups of plants.

By 1926, the knowledge in this field had been extended by Fred, Whiting, and Hastings (23) in a summary of 10 years' study to include eight cross-inoculation groups, whereas Burrill and Hansen (11) distinguished eleven groups, within which species of the host plant are interchangeable for the purpose of inoculation and beyond which nodules cannot be produced. In 1928, Walker (52) listed 18 such groups, 7 of which contained more than one species of host plant, and the other 11, with one host only, were reported as incapable of cross-inoculation with other legumes so far as tested.

During the last decade interest has centered in the discovery of physiological differences in strains within a single cross-inoculation group. The distinctions are based on constant differences in the effects of inoculation on the plant, distribution of nodules over the root system, varying amounts of increased yield and nitrogen content when inoculated with different strains, and finally serological reactions with the organism as antigen.

The last named distinction, being of high specificity, is therefore of the greatest value. To date, six of the cross-inoculation groups have been subdivided by Fred, Whiting, and Hastings (23) on the basis of agglutination tests into from two to four agglutination groups, including strains of the organism which show other similar characteristics, such as variation in the power of nitrogen fixation.

In 1929, Baldwin and Fred (6) published an abstract of some of their current investigations on strain variation in *Rhizobium trifolii* in which 18 strains were tested for efficiency in aiding

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plant growth. Considerable variation was shown in the benefit derived by the host. An interesting variation in nodulation was coordinate with their effectiveness, namely, that more but smaller nodules were formed by the poor strains than the good, and they were distributed over the whole root system. In the good strains, the nodules were fewer, larger, and located on the upper portion of the root system.

Thus, at present, attention is directed toward a study of the efficiency of these distinct strains. Dunham and Baldwin (16), in 1931, tested the effect of double infection with good and poor strains and found that plants already infected with one strain of organism resist the entrance of a contrasting strain to a much greater extent than do nodule-free plants.

It is interesting to note that in most of the work done, an open pot method of cultivating the inoculated host plants has been used. In other experiments small seeds, e.g., clover and alfalfa, have been inoculated and grown on an agar substrate in test tubes, and Wilson, Hopkins, and Fred (55), in 1930, studied the fixation of nitrogen by plants grown on agar in 12-oz. and 32-oz. bottles. Care was exercised to reduce as far as possible the effect of factors detrimental to the physiology of the plant. However, since growth in closed containers, in agar, forces a highly artificial environment upon both the host plants and the invading bacteria, the authors themselves state that it is apparent "that this method is of little value in the comparison of the relative efficiency of strains except in a gross way."

It seemed desirable to test the effectiveness of various strains of *Rhizobium trifolii* under bacteriologically controlled conditions, imitating as nearly as possible the conditions found in nature, especially as they affect root development. To this end various strains were grown on plants of *Trifolium pratense* and *T. hybridum* in sterile sand cultures by a method, the details of which are described later. The ultimate purpose was to obtain tested strains of high efficiency in nitrogen fixation.

## EXPERIMENTAL

### *History of Cultures*

Twenty-two strains of *Rhizobium trifolii* were used in these investigations, seven of which were isolated locally and fifteen of which were "imported strains," obtained in culture from investigators in other universities or state experiment stations. Table 1 gives their history, Roman numerals having been used to indicate local strains and Arabic numerals imported strains.

All cultures have been grown on mannite agar with transfers approximately once a month. The formula for this medium is as follows:

Mannitol .....	20 gm.
Magnesium sulfate .....	0.2 gm.
Di-potassium phosphate .....	0.2 gm.
Sodium chloride .....	0.2 gm.
Calcium carbonate .....	5.0 gm.
Agar .....	15.0 gm.
Distilled water .....	1,000.0 cc.

### *Cultural Characteristics*

The cultures show characteristic differences in growth on mannite agar slants of 2-day-old cultures, incubated at 27°C. The most pronounced differences

TABLE 1  
History of cultures used in experiments

CULTURE NUMBER	SOURCE OF STRAIN	DATE OF ISOLATION
<i>Local strains</i>		
I	Isolated from <i>Trifolium pratense</i> by Plant Physiology Class in Syracuse University, "Clover I"	Nov. 1927
II	Isolated from <i>Trifolium pratense</i> by Plant Physiology Class in Syracuse University, "Clover II"	Nov. 1927
III	Isolated by author from <i>T. pratense</i> collected near East Liverpool, N. Y.	Aug. 1928
IV	Isolated by author from <i>T. hybridum</i> collected near East Liverpool, N. Y.	Aug. 1928
V	Isolated by author from <i>T. repens</i> collected at Skaneateles, N. Y.	Aug. 1928
VI	Isolated from <i>T. pratense</i> by Josiah Lowe, Syracuse University	Nov. 1928
IX	Isolated by author from <i>T. hybridum</i> , Tully, N. Y. (growing in presumably acid soil)	Oct. 1928
<i>Imported strains</i>		
1	"Red Clover 104W" Columbia, Mo. Isolated from exchange culture from University of Wisconsin. Represents five transfers	Mar. 1928
2	"Red Clover 105" Columbia, Mo. Isolated from exchange culture from U. S. D. A.-Leonard. Represents 29 transfers	Mar. 1925
3	"Red Clover 106" Columbia, Mo. Isolated from nodule. Represents 11 transfers	May 1927
4	Isolated from sand cultures supplied by Cornell Exp. Sta., Ithaca, N. Y.	Fall 1928
5	"Trifolium 1" Madison, Wis. Isolated from plants of <i>T. suaveolens</i> . Seed obtained in Germany and grown on new land. Tested 1927	Fall 1927
6	"Trifolium 203" Madison, Wis. Isolated from nodules of <i>T. pratense</i> at Madison. Tested 1921 and 1922. Always positive	1921
7	"Trifolium 205" Madison, Wis. From nodogen culture in 1922. Replated and tested annually 1922-1927. Always positive	1922
8	"Pa. Red Clover" from Penn. State College, pure culture	Transferred Syracuse Aug., 1928
9	"Trifolium #1" Mass. Agr. Col., Amherst, Mass.	Received in Syracuse Sept. 1928
10	"Trifolium #2," Amherst, Mass.	ditto
11	"A. Dickinson Co. Nod-o-gen Clover," Amherst, Mass.	ditto
12	"Mulford Co. 1927 Clover," Amherst, Mass.	ditto
13	"Earp-Thomas Co. Homogerm-Clover," Amherst, Mass.	ditto
14	"Earp-Thomas Co. Farmogerm-Clover," Amherst, Mass.	ditto
15	" <i>Rhizobium radicicola</i> on clover," Dept. of Bacteriology, Univ. of Nanking, Nanking, China	Spring 1930

occur in the abundance of growth and the consistency, since there is decided variation in gum production.

### *Tests for Purity of Cultures*

All cultures were tested for purity before further experimentation was undertaken. The two methods used were those followed by Löhnis and Hansen (41) for distinguishing "*B. radiculicola*" from the most frequent contaminator *B. radiobacter*. The substrata used were milk, and plugs of white potato cut with a slanting surface, sterilized in the autoclave for 15 minutes at 15 pounds pressure. They were then inoculated with a loop of material from the stock cultures.

If the culture tested is "*B. radiculicola*," there will appear at the surface of the milk after 1 to 4 weeks a perfectly clear serum zone 2-5 mm. thick, while the milk below remains nearly unchanged. *B. radiobacter* forms first a slime ring on top and later the whole milk turns brown. On potato, "*B. radiculicola*" forms a meager, transparent, slimy growth, whereas if *B. radiobacter* is present, the potato turns gray frequently and the growth is at first gray but later becomes a coli-brown slimy layer, thus making a sharp distinction between *B. radiobacter* and the nodule bacteria. This growth in milk and on potato is stated to be quite characteristic and can be used to great advantage as a test for purity of culture. The results of these tests showed that five of the cultures; namely, III, IV, 9, 12, and 13, did not give the characteristic reaction for "*B. radiculicola*."

### *Tests for Positivity*

All cultures were then tested for their ability to produce nodules on the host plant. As a growth medium 0.65 per cent agar in tap water was used in test tubes. For inoculum a bacterial suspension from 4-day-old cultures growing on mannite agar slants was made by washing the growth from the agar surface.

Inasmuch as more than half of the cultures had been isolated from red clover, seeds of *Trifolium pratense* were used. These were sterilized by immersion in 1:1,000 mercuric chloride, then thoroughly washed in sterile distilled water, and transferred to the culture tubes with a sterile platinum loop. Inoculation was obtained by adding five drops of the heavy bacterial suspension after the seeds had been placed in the culture tube. The cultures were kept in the dark for 2 or 3 days until the seeds had germinated, then taken to the greenhouse where they were set in sawdust up to the agar level to shield the roots against light. When the cultures were grown in summer, cheesecloth was used to cover and shade them from brilliant sunlight.

Plate 1, figure 1, shows the results of such tests for nodule formation.

Eight cultures showed no nodule formation, III, IV, 4, 5, 11, 12, 13, and 15. The others were all positive and produced abundant nodules in this and in all subsequent tests.

Following the test made in July, 1930, nodules were removed from each of

the positive cultures and the organisms reisolated, the original stock cultures being replaced.

### *Sand Cultures*

A total of 10 sets of clover crops were grown in sand cultures, each crop growing for a period of 70–120 days before being harvested and tested. Two methods of setting up the sand cultures were used, open pot and bacteriologically controlled.

*Open Pot Cultures.*—The open pot method was used in the first three sets only. It is by this method that the majority of previous investigators have carried out their experiments. It consisted in the preparation of 1-gallon earthenware jars as culture chambers. These were filled with glacial sand which was very low in nitrogen content. The pH of the sand used was 8.2–8.4.

It was determined that 400 cc. of water per jar was necessary to bring the sand to the proper moisture content for germination and growth. This was added before sterilization, and heavy paper was tied tightly over the top of each jar. They were then autoclaved at 15 pounds pressure for 18 hours—6 hours for each of three consecutive days. After removal from the autoclave, they were cooled to room temperature before the seeds were planted. If the surface of the sand appeared dry after sterilization, sterile distilled water was added as required.

Viable red clover seeds, *Trifolium pratense*, were used. Sterilization of seeds was by immersion in 1:1,000 mercuric chloride as previously described. The same number of seeds was placed on the surface of each jar and it was immediately inoculated by pouring over it a bacterial suspension made from 4-day-old mannite agar cultures of one of the strains of *Rhizobium trifolii*.

The jars were then taken to the greenhouse and kept in a separate section where every precaution was taken to guard against contamination.

A sterile nutrient solution was prepared according to the formula used by Helz, Baldwin and Fred (31) as a "Modification of Bryan's." The following salts were ground together in a mortar to obtain a homogeneous mixture.

	gm.
Potassium chloride.....	10
Calcium sulfate.....	2.5
Magnesium sulfate.....	2.5
Calcium phosphate.....	2.5
Iron phosphate....	2.5
Potassium phosphate, tribasic.....	2.5

The nutrient solution was made up on the basis of 3 gm. of mixed salts per liter, and 100 cc. of the solution was added to every jar every 2 weeks. If more moisture was needed, the plants were watered with distilled water only.

Sets I, II, and III each contained 22 such cultures, with 2–5 checks per set, i.e. cultures prepared in the same way except that they were not inoculated.

In harvesting the crops, the plants were carefully washed free of sand—sur-

plus water being removed—, placed in paper bags, and labelled. These were thoroughly dried in an oven kept at 90°C. for 24 hours, then at 105°C. for several days. Then each crop was reduced to a fine powder and placed in a tightly stoppered bottle.

Before the entire crop was weighed, the powdered material was again placed in an electric oven kept at constant temperature of 105°C. for a day, allowed to cool in a Frühling-Schultz desiccator, and then weighed.

For determination of the nitrogen content a modification of the modified Koch-McMeekin method (28) for micro-Kjeldahl tests was used. A 20-mgm. sample of the dried clover powder was placed in a chemically clean and dry Pyrex test tube graduated to 50 cc. To this was added for digestion 2 cc. of sulfuric acid, 1:1 dilution + 5 per cent copper sulfate, and the mixture boiled vigorously over a micro-burner until, after blackening, the characteristic dense white fumes began to fill the tube. At this time a watch glass was placed over the mouth of the tube and the flame was immediately reduced to an amount just sufficient to keep the mixture boiling gently for 5 minutes. To reduce the time necessary for digestion a few drops of Superoxol were then added, the burner having been moved aside so that the acid was not actively boiling. Better results are obtained if the Superoxol is dropped directly into the hot solution from a capillary pipette and not allowed to run down the side of the tube. Enough Superoxol is necessary to change the color of the mixture from dark brown to colorless or a light straw-color,—thus acting as a strong catalyst. Again the material is boiled gently for 5 minutes. If the solution discolors, an additional drop or two of Superoxol will clear it. After cooling, approximately 5 cc. of distilled water was added to the colorless solution and it was ready for distillation.

After the work of nitrogen determinations had been completed, it was noted that Sullivan and Horat (49), in 1929, had suggested that dry plant material may be digested in a test tube with the aid of Superoxol and the resulting analysis be of as high accuracy as the regular Kjeldahl method. In their microchemical tests, however, they used repeated additions of Superoxol, five drops at a time, and after the mixture cleared, boiling for 3 hours to complete digestion. An aeration process requiring 2 hours followed before the material was ready for titration.

In the method used in this investigation from 5–20 drops of Superoxol was added after the first 5-minute period of boiling, the number of drops depending on the amount necessary to clear the mixture, and thereafter digestion was completed with boiling for only 5 minutes more. The subsequent distillation required about 5 minutes, thus making the entire process previous to Nesslerization, a matter of about 20 minutes for each sample.

At this time the total plant nitrogen has been digested and changed to ammonium sulfate. The Koch-McMeekin method calls for direct Nesslerization but with the samples of clover material, this frequently resulted in a cloudy liquid which could not be used for comparison against a standard in a

colorimeter. Therefore it was found necessary to distill over the nitrogen into sulfuric acid.

Distillation consisted in first neutralizing the material by addition of a sufficient amount of a concentrated solution of sodium hydroxide, (410 gm. to 1 liter). The test tube had previously been connected with a small Liebig condenser which led into a 50-cc. volumetric flask containing 5 cc. of .1 *N* sulfuric acid and 10 cc. of distilled water. On the addition of the sodium hydroxide, the ammonium sulfate is changed to ammonium hydroxide, and when the test tube is heated with a microburner this becomes volatile and is distilled over into the flask containing the acid, where it again becomes ammonium sulfate, but all colloidal matter has been left behind and the liquid on Nesslerization remains crystal-clear. The heating usually requires from 3 to 5 minutes before crystals begin to form in the tube, which indicates the end point.

Figure 1 shows the arrangement of the apparatus used for distillation.

The material in the flask is then Nesslerized by the slow addition of 10 cc. of the Koch-McMeekin Nessler's solution, with constant shaking, and the mixture made up to 50 cc. with distilled water. It is then ready to be colorimetrically compared with a standard.

The standard is made up by adding to 5 cc. of ammonium sulfate, adjusted to contain 0.3 mgm. nitrogen in 5 cc., approximately 10 cc. distilled water, then 10 cc. Nessler's, and enough more water to make it up to 50 cc. A single standard can be used safely for readings for half an hour only, during which time a number of unknowns may be compared with it.

The Klett bio-colorimeter was used for these determinations and from the readings obtained, the milligrams of nitrogen and the percentages of nitrogen were calculated. Duplicate tests were run for each culture and the average of five colorimeter readings was taken for each sample. If there was an appreciable discrepancy in the average readings of the two samples, one or more samples were retested before further calculations were made.

*Bacteriologically Controlled Cultures.*—After a study of the results of sets I, II, and III it became obvious that the open-pot method was not satisfactory for a study of the effects of different strains of bacteria, since nodules were found on the roots of all cultures, including controls as well as the ones inoculated with strains which were non-nodule-producing in agar culture. The open-pot method was therefore abandoned, and it became necessary to devise a method which would supply bacteriologically controlled conditions, and at the same time be as nearly similar to natural conditions as possible.

In the last seven sets a modification of the apparatus suggested by Deatrick (15) was used, which in turn included an adaptation of an arrangement for keeping seeds sterile, suggested by Fred (20). The method makes it possible to supply sterile nutrient solution as required and still keep the jar covered and the plants protected against contamination.

Figure 2 illustrates the arrangement of the apparatus.

A Livingston auto-irrigator cone was buried in the sand in each jar. The



auto-irrigator is made of unglazed clay and therefore when filled with nutrient solution readily supplies moisture to the surrounding sand. In the 2-holed rubber stopper in the neck of the cone, a 10-cc. pipette was inserted to serve as a safety reservoir when filling the cone, and also a bent glass tube leading over the edge of the jar, to which the flasks of nutrient solution were connected.

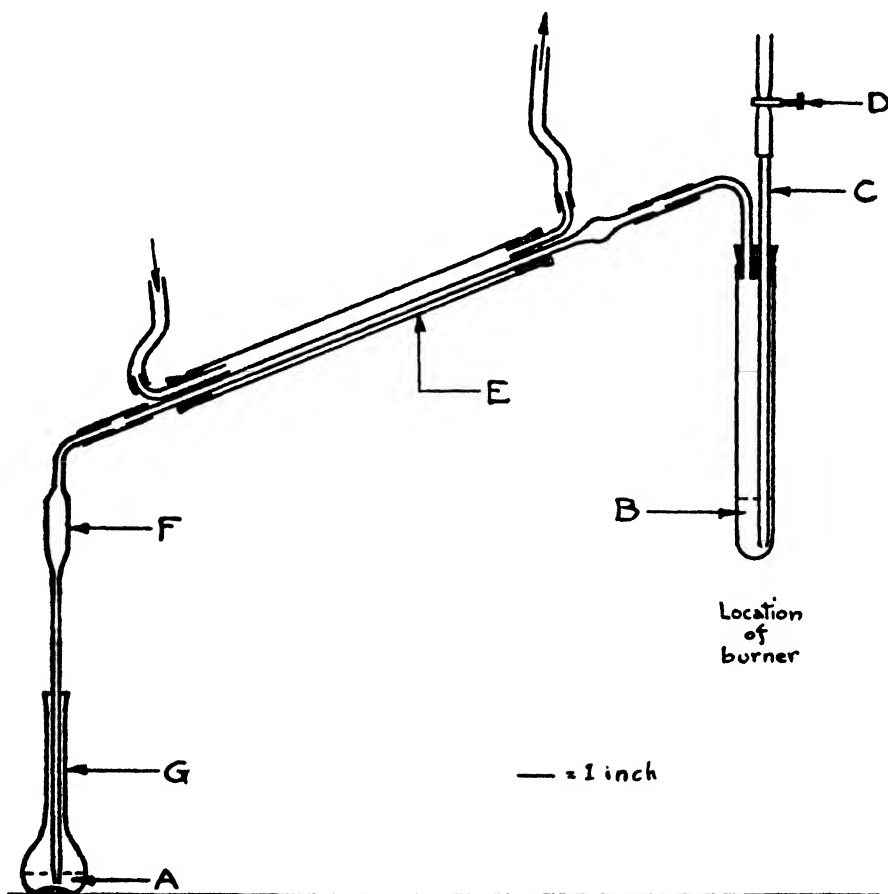


FIG. 1. ARRANGEMENT OF APPARATUS FOR DISTILLATION

A, .1 *N* sulfuric acid; B, digested material; C, tube for holding NaOH; D, clamp on rubber tubing for introduction of NaOH; E, Liebig condenser; F, safety reservoir; G, Volumetric flask.

This arrangement supplies sterile nutrient directly through sterilized containers and tubes, preventing any possible contamination.

By mixing a similar quantity of sand with enough water to bring it to the proper consistency for planting, the amount to be added to each jar was determined. Just before this was added, approximately 5 gm. of powdered calcium

carbonate was mixed with the sand to insure an excess of lime which favors the growth of the root-nodule organisms.

After the addition of the water, two Pyrex glass cylinders,  $1\frac{3}{4}$  inches in diameter and 8 inches tall were set upright on the surface of the wet sand, which stood at a level about an inch below the top of the jar. They were held in place by fine, washed gravel which was filled in around the tubes and reservoir pipette. A heavy layer of non-absorbent cotton was placed over the surface of the gravel, which served to keep the cotton from becoming wet through absorbing moisture from the sand. Then the whole jar was tightly

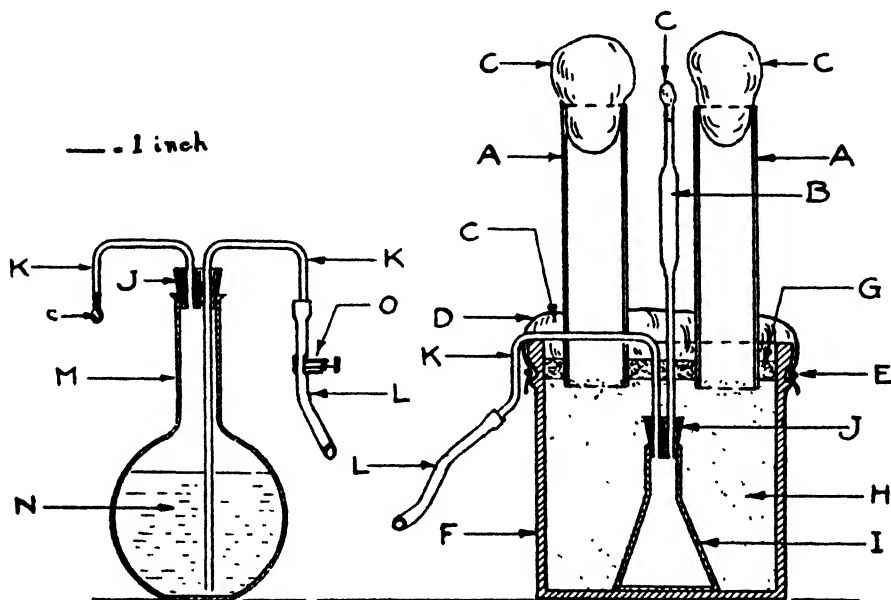


FIG. 2. APPARATUS FOR BACTERIOLOGICALLY CONTROLLED CULTURES

A, Pyrex glass cylinders—plant chambers; B, 10-ml. pipette-reservoir; C, cotton—non-absorbent; D, waterproofed paper; E, string; F, 1-gallon earthenware jar; G, washed gravel; H, glacial sand; I, Livingston Auto-irrigator cone; J, two-holed rubber stopper; K, bent glass tube; L, rubber tubing; M, 1-liter Pyrex flask; N, sterile nutrient solution; O, screw clamp.

covered with heavy waterproof paper, holes being cut in it, which fitted closely around the cylinders and the pipette, and also fitting over the bent tubes from the irrigator at the edge of the jar.

Cotton plugs were then fitted into the large glass cylinders which served as plant chambers and also small plugs closing the top of the pipette and the end of the irrigator supply tube.

At this stage each jar was weighed and numbered so that after sterilization enough sterile nutrient solution could be added to bring it up to its original weight and optimum condition for planting. The jars were then autoclaved at 15 pounds for 18 hours of intermittent sterilization.

The nutrient solution to be used in these sets was made up in 1-liter flasks, 100 cc. of a stock nutrient solution being used to 900 cc. distilled water. The stock nutrient contained 6 gm. of the mixed salts previously described, to 1 liter of distilled water. In each flask was placed a 2-holed rubber stopper fitted with two bent glass tubes, one extending to the bottom of the flask and the other a short bend with cotton plug in the free end. To the longer one was attached about 3 feet of rubber tubing. The end of this tubing was wrapped in paper, held with a paper clip. These flasks were then sterilized in the autoclave at 15 pounds for 15 minutes.

After the sterilized culture jars had cooled, (half a day in the autoclaves and over night outside), they were weighed again and the loss of weight in sterilization was made up by the addition of nutrient solution through the rubber tube on the flask which was connected to the irrigator supply tube on the jar. This brought the sand again to optimum moisture conditions.

Throughout the growth of these controlled sets, the jars were kept at constant weight by the addition of nutrient solution at weekly intervals.

Ten seeds, sterilized in mercuric chloride, were dropped to the surface of the moist sand in each Pyrex cylinder from a flamed platinum loop and the cotton plug immediately replaced. The inoculum was made up in sterile water from two or three tubes of 5-day-old mannite agar slant cultures, half of the bacterial suspension being poured over the seeds in each cylinder. As a final precaution against contamination, the paper covering of the culture was thoroughly sprayed with shellac.

Figures 2 and 3 of plate 1 show the arrangement of the cultures after they had been taken to the greenhouse. The harvesting of the crop and the determination of dry weights and nitrogen content were carried out as previously described.

Sets IX and X were limited to those cultures which had been rather consistently "best" or "poorest," and they were grown at the same time. Planted and inoculated March 30, 1931, reinoculated May 4, they were harvested July 17. Strains I, VI, IX, and 9 were selected as "very good" strains, and V, 6, and 7 as "very poor."

Plate 2 shows contrasting growth in bacteriologically controlled cultures.

Tables 2 to 5 show the comparison in the development of the crops in the different sets, when inoculated with the various strains of *Rhizobium*. Set II, with open-pot cultures, is included, since it shows wide variations from the controlled sets, in many instances. The measurement of tops and roots of the clover plants at harvest time was not begun until the fifth set.

### *Field Plots*

Since the 10 sets of cultures grown on sterilized sand indicated that inoculation with certain strains would, with reasonable consistency, produce a very good crop, whereas others would show only a fair or poor growth, it was decided to try out these strains in the field.

TABLE 2  
*Summary of appearance of crops*

CULTURE NUMBER	SET II*	SET III*	SET IV	SET V	SET VI	SET VII	SET IX	SET X
I	Medium	Medium	Very good	Very good	Very good	Medium	Poor	Poor
II	Medium	Medium	...	Medium	Very good	Very good	...	...
III	Very good	Medium	...	...	...	...	...	...
IV	Very good	Very good	...	...	...	...	...	...
V	Poor	Very good	Poor	Fair	Fair	Fair	Fair	Fair
VI	Poor	Very good	Very good	Very good	Very good	Very good	Medium	Very good
IX	Medium	Medium	Very good	Very good	Very good	Medium	Fair	Very good
1	Medium	Medium	Medium	Medium	...	Very good	Fair	Very good
2	Medium	Poor	...	...	...	...	...	...
3	Medium	Medium	Very good	Fair	...	Very good	...	...
4	Poor	Medium	Poor	...	...	...	...	...
5	Medium	Poor	...	...	...	...	...	...
6	Medium	Medium	Medium	Medium	Medium	...	...	...
7	Poor	Medium	Medium	Poor	Poor	Fair	Very good	Very good
8	Medium	Very good	Very good	Medium	Fair	Fair	Fair	Fair
9	Medium	Poor	Very good	Medium	Very good	Poor	...	...
10	Medium	Medium	Medium	Very good	Very good	Medium	Very good	Very good
11	Very good	Poor	Medium	Medium	Medium	...	...	...
12	Medium	Poor	Poor	Poor	Poor	...	...	...
13	Medium	Medium	...	...	...	...	...	...
14	Very good	Medium	Very good	Medium	Medium	Fair	...	...
15	...	...	...	Poor	Poor	Poor	...	...
A	Medium	Poor	...	Medium	Medium	Fair	...	...
B	Medium	Very good	Poor	Poor	Poor	Poor	Poor	Poor
C	...	Poor	Poor	Poor	Poor	Poor	...	Poor
D	...	Poor	...	Poor	Fair	Poor	...	...
E	...	Medium	...	...	Poor	Poor	...	...

\* Open pot cultures; the others were bacteriologically controlled.

TABLE 3  
Measurement and nodulation of crops V, VI, VII, IX, and X

CULTURE NUMBER	SET V			SET VI			SET VII			SET IX			SET X			AVERAGE MEASUREMENTS	
	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots
I	6.25	6.25	a-k-u	4	4	u	3.25	8.75	c-m-u	2.75	10	d-s-u	3.5	8	.....	4	7.4
II	5	5	d	4	5	l-s-u	5.25	9	a-l-u	.....	.....	.....	.....	.....	.....	4.8	6.3
V	4.25	4.75	a-l-w-u	4	3	a-l-s-u	3.5	7.5	b-m-s	3	7	b-l-w	2.25	5.5	.....	3.4	5.9
VI	4	6	s-k	4.5	4.5	k-u	4	8	l-u	5	9.5	a-m-s	7	9.5	.....	4.9	7.5
IX	4.25	4.25	k-u	4.5	4	u	3	8	m-s	3.5	7.5	k-s-u	4.5	7.5	.....	4	6
1	4	5	d	.....	.....	.....	4	8.5	a-m-s-u	.....	.....	.....	.....	.....	.....	4	6.8
3	4.5	5.5	d-k-u	.....	.....	.....	4.5	7	a-l-w	.....	.....	.....	.....	.....	.....	4.5	6.3
6	3.5	7	d-s	3.5	5	a-s	3	8	k-s-v	3.5	6.5	k-s-u	4	8	.....	3.5	6.9
7	2	5	b-k-t	1.75	2.75	b-k-s-t	3	7.5	m-v	4	9	a-m-t	3	7	.....	2.8	6.3
8	3.5	6.5	None	1.75	5	none	1.5	4.5	none	.....	.....	.....	.....	.....	.....	2.3	5.3
9	4.5	4.5	a-s	4	4.5	a-l-s-u	3	5.5	d-l-u	4.5	7	u	5.5	8.5	.....	4.3	6
10	6	6	l-s-u	3.5	4.5	a-s	3.5	6.5	l-w-v	.....	.....	.....	.....	.....	.....	4.3	5.7
12	2	6	none	2	4	none	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	5
14	4.5	2.5	d-k-u	4	4	d-l-u	3	9	d-m	.....	.....	.....	.....	.....	.....	3.7	5.2
15	2	5	none	3.5	4.5	d-k-v	1.5	6.5	d	.....	.....	.....	.....	.....	.....	2.3	5.3
A	2.25	7	none	2	3.5	none	1.5	3.5	none	2	10	none	2.5	7.5	none	.....	.....
B	2.25	8	none	2.25	5	none	1.5	5	none	.....	.....	.....	2.5	7	none	2.14	6.5
C	3.75	6.25	none	2.5	5	none	2	7	none	.....	.....	.....	.....	.....	.....	.....	.....
D	.....	.....	.....	1.5	7	none	2.25	9.75	none	.....	.....	.....	.....	.....	.....	.....	.....

\* The key to the description of nodulation is as follows: a, abundant; b, very abundant; c, fairly abundant; d, few; k, small; l, large; m, medium; n, all sizes; s, scattered; t, on all roots; u, on upper roots; v, on smaller roots; w, well scattered.  
All measurements in length of tops and roots are in inches.

It must be remembered that in the microbiological equilibrium of the soil in nature, *Rhizobium* is only one of many microorganisms living in a given location and, as such, must maintain itself against competition in sufficient numbers and with enough virulence to be effective in nitrogen fixation.

TABLE 4  
*Total nitrogen in crops*

Set No.....	II	IV	V	VI	VII	IX	X	AVERAGE FOR SETS* V, VI, VII, IX, X
Duration of growth.. ....	4 mos	2½ mos.	3 mos.	3 mos.	4 mos.	3½ mos.	3½ mos.	
Season.....	Feb.-May	June- Aug.	Aug.- Nov.	Sept - Dec	Jan.-May	Mar.- July	Mar.- July	
Culture number	Total nitrogen in mgm. in entire crop							
I	175.58	2.56	6 13	6.52	3.87	5.00	5.74	5.45
II	173.55	.....	5 44	5.26	5.06	.....	.....	5.25
III	185.48	.....	.....	.....	.....	.....	.....	.....
IV	148.79	.....	.....	.....	.....	.....	.....	.....
V	70.45	1.01	3 67	5.62	3.75	3.93	5.41	4.48
VI	77.38	1 94	5 36	5.80	4.75	6 45	6.64	5.80
IX	190 68	1 40	5 63	7 47	3.46	10 37	7.38	6.86
1	99 28	2.57	4 42	.....	5.78	.....	.....	5.10
2	103 62	.....	.....	.....	.....	.....	.....	.....
3	159 49	1 64	4 22	.....	4 54	.....	.....	4.38
4	185 08	0 58	.....	.....	.....	.....	.....	.....
5	123.47	.....	.....	.....	.....	.....	.....	.....
6	126 16	1.88	6 16	6.76	2 23	5 32	6.26	5.35
7	85 85	1.57	2 32	3 48	2 03	5 20	6 00	3.81
8	103.10	1 51	3 27	3 82	1.97	.....	.....	3.02
9	154 82	1.57	1.15	4 75	4 38	8 04	7.52	6.17
10	76 72	1 15	5 06	5 74	3 73	.....	.....	4.84
11	185 41	0 90	.....	.....	.....	.....	.....	.....
12	159.95	.....	1 46	4 72	.....	.....	.....	3.09
13	145.51	.....	.....	.....	.....	.....	.....	.....
14	232.04	1.87	5 82	5.83	3.84	.....	.....	5.16
15	.....	.....	1 52	2 49	1.32	.....	.....	1.78
A	83.11	0.58	1 57	4.19	0.65	2.75	4.61	2.35
B	158.51	1.06	1 59	3.63	2.19	.....	4.79	3.05
C	.....	.....	1.65	3 72	1 85	.....	.....	2 61
D	.....	.....	.....	2 56	2.79	.....	.....	2.67

\* Set II, being an open pot set, was omitted from the average.

Set IV was omitted from the average because its shorter growth period made it incompatible with the others.

Very small experimental plots were prepared in a cherry orchard between the rows of trees, the space having been previously covered with grass. The plots were 1 foot square; they were carefully spaded and prepared, free of larger roots, and were kept weeded. Five grams of seeds, (approximately 100) of *Trifolium pratense* were planted in each plot, the seeds having been first

inoculated by soaking 5 hours in a bacterial suspension made from two tubes of 5-day-old cultures. Six very good cultures and three poorer ones were chosen as the strains to be tested, and three control plots, uninoculated, were planted, the seeds having been first soaked in water. Three months after planting, the following observations were made on the development of the

TABLE 5  
*Percentage nitrogen in crops*

CULTURE NUMBER	SET II	SET IV	SET V	SET VI	SET VII	SET IX	SET X	ALL SMALL CROPS, AVERAGE PER CENT N
I	1.54	1.41	1.51	1.99	0.95	1.07	1.23	1.36
II	1.64	.....	1.33	0.79	1.02	.....	.....	1.05
III	1.24	.....	.....	.....	.....	.....	.....	.....
IV	0.97	.....	.....	.....	.....	.....	.....	.....
V	0.90	0.88	1.16	1.78	0.74	0.80	0.49	0.98
VI	0.98	1.55	1.37	1.37	1.24	1.09	1.39	1.37
IX	1.33	1.53	1.49	1.85	1.20	1.86	1.05	1.47
1	0.67	1.74	1.22	.....	1.11	.....	.....	1.36
2	0.87	.....	.....	.....	.....	.....	.....	.....
3	0.90	0.83	1.47	.....	1.00	.....	.....	1.10
4	1.08	0.75	.....	.....	.....	.....	.....	0.75
5	1.26	.....	.....	.....	.....	.....	.....	.....
6	0.98	1.62	1.60	1.26	0.77	0.86	0.97	1.18
7	1.13	1.71	0.89	0.73	0.80	0.99	0.92	1.00
8	1.04	1.30	0.91	0.58	0.61	.....	.....	0.85
9	1.27	1.51	1.17	1.33	0.81	0.94	0.99	1.13
10	0.54	1.27	1.33	1.57	1.31	.....	.....	1.40
11	1.13	0.88	.....	.....	.....	.....	.....	0.88
12	1.36	.....	0.43	0.84	.....	.....	.....	0.64
13	0.93	.....	.....	.....	.....	.....	.....	.....
14	1.56	1.92	1.33	1.14	0.88	.....	.....	1.32
15	.....	.....	0.46	0.83	0.80	.....	.....	0.70
A	0.69	0.52	0.79	0.64	1.13	0.39	0.34	0.72
B	1.14	0.63	0.48	0.83	1.15	.....	0.50	
C	.....	.....	0.83	1.17	0.81	.....	.....	
D	.....	.....	.....	0.81	0.64	.....	.....	

crop, based on amount of growth, color and size of leaves, and general condition of the plants.

Plate 3 shows some of the field plots in this test.

#### *Agglutination tests<sup>3</sup>*

Since the serological differentiation is the most specific means of separating physiologically distinct strains, the next step was to test all strains for their agglutination reactions.

<sup>3</sup> This portion of the work was carried out by Miss Glenna Wurth, under the direction of the author.

Five antisera were obtained from rabbits following injection with five selected strains of bacteria as inocula. Cultures, VI, IX, and 9 were chosen as consistently good strains, and cultures V and VII as poor ones. The sera were tested with their homologous antigens (suspensions of young cultures of the bacteria used for injection) and produced agglutination. When tested against all the other strains, no agglutination occurred.

Normal blood serum from an uninoculated rabbit was tested against each of the five antigens used for inoculation and showed no agglutination.

From these results it can be concluded that with the five sera used, six different agglutination groups are indicated, each serum agglutinating only its homologous antigen, and those not agglutinated constituting a sixth group. The fact that there is no cross-agglutination leads to interesting speculations as to what might have happened had sera for all 14 strains been available and suggests the possibility of demonstrating as many as 14 groups—one for each

TABLE 6  
*Field test*

CULTURE NUMBER	RATING BEFORE TEST	GROWTH IN THE FIELD
I	Very good	Very good
II	Very good	Very good
V	Fair	Medium
VI	Very good	Very good
IX	Very good	Good
3	Very good	Very poor
6	Poor	Poor
7	Poor	Poor
9	Very good	Best
A		Very poor
B		Fair
C		Fairly poor

strain. The results do seem significant, however, in that each strain reveals the serological characteristics ordinarily associated with a distinct species.

#### DISCUSSION OF RESULTS AND CONCLUSIONS

The open-pot method for testing the efficiency of different strains of bacteria was proved unreliable, since *all* cultures, even controls and those inoculated with negative strains, showed the presence of nodules. The comparison of growth in the three sets of open-pot cultures shows very inconsistent results in many cases, even to one check culture in set III producing as fine a crop as any inoculated culture.

The bacteriologically controlled cultures can be relied upon to give results without contamination. In none of the 20 controls grown in connection with the various controlled sets was there a single nodule produced and growth was generally poor. Therefore, the results obtained from inoculation with any given culture can be interpreted as due to that strain alone.



These bacteriologically controlled cultures show rather consistent variations suggesting the existence of physiologically different strains of *Rhizobium trifolii*. Certain strains stand out as having stimulated very good growth throughout most of the controlled cultures,—for example, IX, VI, and 9. Others have been consistently poor, or at best only fair, as V and 7.

A close observation of the growth of these organisms on agar shows cultural differences correlated with the different strains. The "fast growers" and those producing larger quantities of gum seem to be more efficient in their relations to the host plant, whereas the poorer strains produce little gum and a scanty slow growth.

A rather consistent contrast in the nodulation and measurements of roots and tops of the better and poorer strains is apparent. A study of table 3 at this point shows that the more efficient strains have a ratio of from 1:1 to 1:2 in the height of tops to length of roots, whereas the poorer strains and checks have roots that are at least twice as long as the tops. This excessive develop-

TABLE 7  
*Efficiency in nitrogen fixation*

	AVERAGE TOTAL NITROGEN	N
	mgm.	per cent
For all check cultures in controlled sets . . . . .	2 75	0 72
For several negative cultures . . . . .	1.78-3 02	0 70-0 88
For two notably poor strains . . . . .	3 81-4 48	0 98-1 0
For the better strains . . . . .	5.25-6 86	1.13-1.47

ment of the root system seems to be correlated with absence of nodules or infection by an ineffective strain of bacteria.

In nodulation a consistent difference is shown in that the poorer strains have the usually small nodules thoroughly scattered over the entire root system, whereas in the better strains the nodules are larger and distributed chiefly on the upper roots. This variation in distribution of nodules has also been noted by other observers.

The efficiency in nitrogen fixation shows fairly consistent variation, both in the total nitrogen content of the crop and in the percentage of nitrogen. The contrast in efficiency of cultures is brought out in table 7.

The "efficient strains" maintained a high efficiency throughout the sets, and the poorer, less efficient strains remained so. This is contrary to the result described by Allen and Baldwin (1) following a plant passage, though they pointed out that the changes in efficiency were more marked after several passages and in this investigation there was only one between sets IV and V.

The experiments with field plots, although small in number, give a definite suggestion as to the possibilities of inoculation in nature's dynamic habitat, the soil; namely, that inoculation of seed with the various strains yields results corresponding to those obtained under bacteriologically controlled conditions.

The efficient strains were able to maintain themselves against competition and greatly increase the yield of the crop. The poorer strains were unable to compete successfully, and the crops were even somewhat poorer than the uninoculated controls.

This close correlation suggests therefore that the bacteriologically controlled conditions as supplied in sets IV-X constitute an efficient method of testing out the differences in strains, differences which would presumably maintain themselves in inoculation of seed for growth in the field.

The aforementioned set of experiments suggests that efficient strains, such as 1, 9, 14, I, II, VI, IX could be used for inoculation of *Trifolium* with a definite probability of highly increasing nitrogen fixation. Of these very good strains, IX has proved to be the most efficient, increasing the total nitrogen content of the crop 149.4 per cent over the uninoculated controls. Dunham and Baldwin (16) have shown that increased plant growth results from inoculation of seed with an effective strain even when it is sown in soil carrying an ineffective strain. On the other hand, inoculation of seed with an ineffective strain is detrimental to plant growth, even if the seed is planted on soil already carrying an effective strain of the organism. They further state that the results of these studies indicate the necessity of using for seed inoculation only effective strains of the nodule organism, since definite detrimental results may occur from the use of ineffective strains. It is probable that many of the favorable results obtained from the use of inoculated seed on old land are due to the introduction of more effective strains of the organism; also that many of the failures to produce increased plant growth by seed inoculation are due to the use of ineffective strains of the organism.

#### ECONOMIC BEARING OF THE PROBLEM

That the need of increasing the yield and nitrogen content of leguminous crops in the United States is a real problem was emphasized by Pieters (42) in 1924, when he showed that the culture of red clover in the East is gradually declining. Lipman (40) considered the problem of nitrogen fixation under field conditions so vast and many sided as to assume the proportions of a major factor in our national economy. His suggestion for solution of the problem was that more efficient types of symbiotic legume bacteria, as well as non-symbiotic nitrogen-fixing organisms, must be sought, since commercial nitrogen can supply only a small fraction of the nitrogen requirement of crops.

To this end, commercial cultures of *Rhizobium* are available to agriculturalists for inoculation of seed, but unfortunately, the predicted result has not always followed. Moreover, various commercial preparations have been declared "valueless," "of no practical value," "not satisfactory, and failed to produce nodules" after repeated tests. Among this number have been "Nitro-bacteria soil vaccine" (37), "Soilvita" (18), and "Nitrobion" (38).

It is interesting to note at this point that of the "imported strains" in the foregoing investigation, five of the fifteen were from commercial culture pre-

parations. A study of preceding tables reveals the significant data, given in table 8, regarding these commercial cultures.

When these results are compared with those for uninoculated check cultures in the controlled sets in which the total nitrogen was 2.75 mgm., or 0.72 per cent, and with the nitrogen content of crops inoculated with culture IX, the most efficient strain, showing a fixation of 6.86 mgm., or 1.47 per cent, nitro-

TABLE 8  
*Summary of data on commercial cultures*

CULTURE NUMBER	HISTORY	POSITIVITY	GROWTH	NODULATION	NITROGEN	
					mgm.	per cent
7	From Nodogen Culture in 1922, replated and tested 1922-27 and always found positive. A subculture from "Trifolium 205" from Madison, Wis., designated by Wilson, Hopkins, and Fred (55) as a "good nitrogen-fixer"	All but one test + i.e. 4+	Consistently poor to fair	Very abundant small nodules on all roots (controlled sets)	3.81	1.0
11	"A. Dickinson Co. Nod-o-gen Clover" received from Amherst	4 tests —	Principally poor	Very few on upper roots (open pot set)	0.90	0.88
12	"Mulford Co. 1927 Clover"—from Amherst	4 tests —	Poor	None in controlled sets	3.09	0.64
13	"Earp-Thomas Co. Homogerm-Clover"—from Amherst	All tests —	Medium in open pot sets	Few to fairly abundant in open pot sets	...	...
14	"Earp-Thomas Co. Farmogerm Clover"—from Amherst	All tests +	Very good to medium	Few, medium to large, on upper roots (controlled sets)	5.16	1.32

gen, it becomes evident that strains of *Rhizobium* are available which are more efficient than some of those being supplied commercially. Even the two more efficient of these five commercial strains, 7 and 14, show an *increase* in total nitrogen of only 38 per cent and 87 per cent respectively over the controls, whereas IX could be expected to yield 149.4 per cent more nitrogen to the crop than would be present if it were uninoculated.

It would seem, therefore, that a real economic service might be rendered by obtaining *tested* strains for which such quantitative data for crop improvement have been determined under bacteriologically controlled conditions. The fact that certain strains have consistently maintained themselves in field tests also proves this to be a practical and effective method for determination of efficient strains of *Rhizobium trifolii*.

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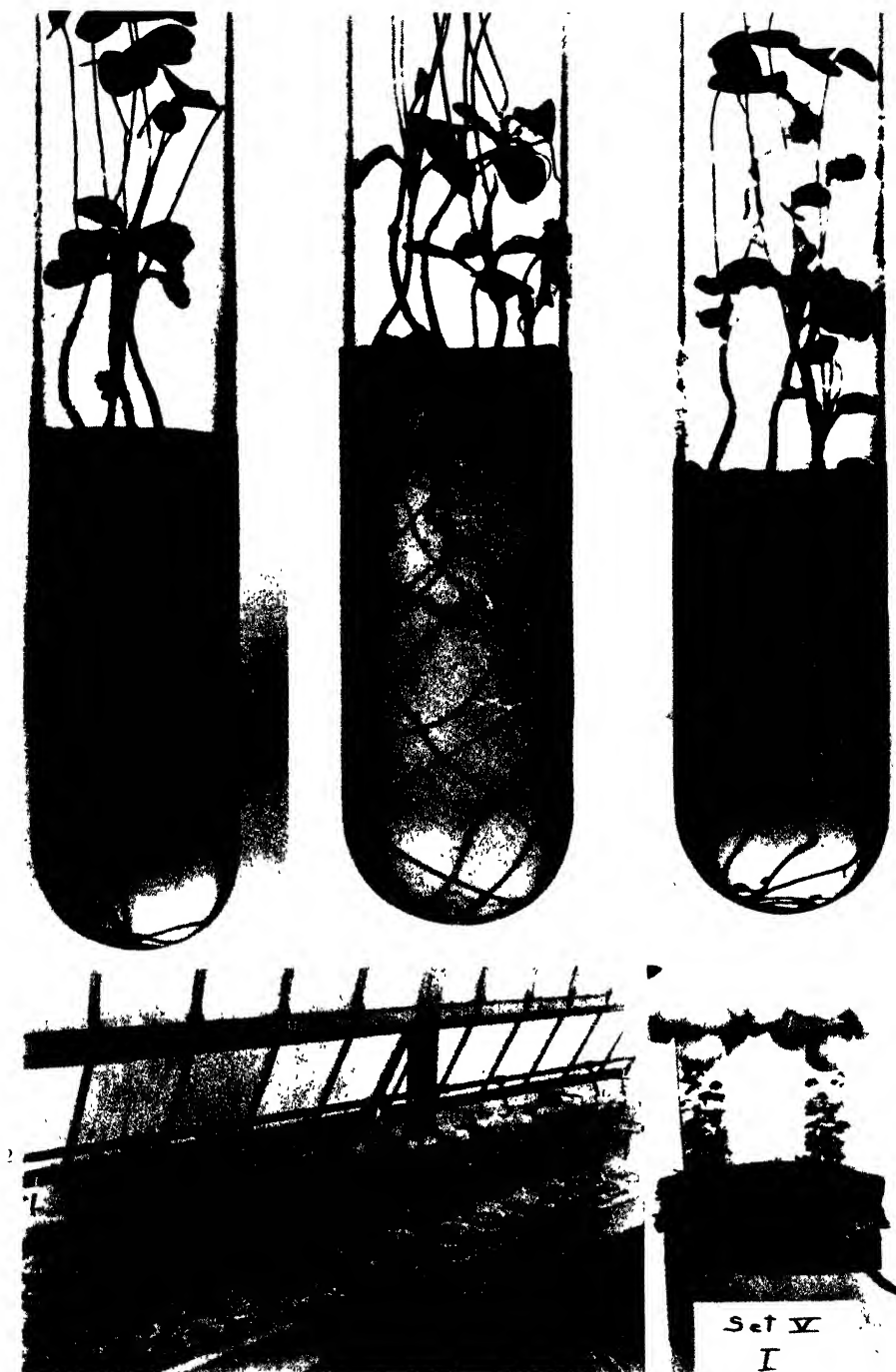
## PLATE 1

## CULTURES OF RHIZOBIUM TRIFOLII

FIG. 1. Test tube cultures showing nodule production with the cultures IX, I, and VI, all locally isolated strains.

FIG. 2. General set-up of cultures in the greenhouse.

FIG. 3. An individual culture.



Figs. 1-3



## PLATE 2

## CONTRASTING STRAINS AS TO GROWTH OF CULTURES IN SEA X

FIG. 1. Comparison of strains V and 9

FIG. 2. Comparison of strains VI and 7.

FIG. 3. A very good culture, VI, compared with a check, A

FIG. 4. A very poor culture, V, compared with a check, B



Figs. 1-4

## PLATE 3

## FIELD PLOTS OF RED CLOVER

- FIG. 1 Plot inoculated with culture II    Measuring stick is in inches  
FIG. 2 Plot inoculated with culture V  
FIG. 3 Plot inoculated with culture I  
FIG. 4 Uninoculated control



FIGS. 1-4



# PRELIMINARY STUDIES IN THE USE OF NITRATE OF SODA ON CERTAIN INDIANA SOILS<sup>1</sup>

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Nitrate of soda has been recognized as one of the standard nitrogen carriers for over a century. Its value as a fertilizer has been tested in comparison with other nitrogenous fertilizers, but definite information on the effects of the time and rate of application upon the yield and quality of the grain is limited.

Nitrate of soda has been commonly used for many years. Kimberly (10), in 1839, wrote: "Saltpeter was known and used as long since as the time of Virgil and we find notice or hint of the effect of nitre or nitrous water worth the attention of farmers in the Sylva of the Bacon, published in the year 1670." He likewise relates various experiments in which the English farmers had tested nitrate of soda down to 1828. The teachings of Liebig (11) caused considerable opposition to the practice of using nitrate. However, this period of conflict stimulated a more thorough test of the material and by the latter part of the nineteenth century nitrate of soda was accepted as a superior source of nitrogen.

Nitrate fertilization was introduced into the United States about the latter half of the nineteenth century, and as a result various experiments were established to determine its value as a fertilizer. Very favorable increases were obtained by McBryde (12) from its use as a side-dressing for corn and by Hayward (8), as a top-dressing for wheat. These tests were then followed by the work of Voorhees (18), who obtained a substantial increase from a 100-pound application on timothy, and by Atwater and Phelps (1), who obtained net profits of \$3.00, \$6.60, and \$4.47 when they applied nitrate at the rate of 25, 50, and 75 pounds to the acre, respectively.

Patterson (15), Duggar and Williamson (5), Moores and Roberts (13) tested the value of nitrate on corn and they concluded that the highest yields were obtained when the nitrate of soda was applied as a divided application, one-half at planting time and the remaining half at the stage of most active growth.

Bizzell and Morgan (2), Taylor (17), Hutcheson (9), and Harvey (7) reported experiments on the value of nitrate on grass land and concluded that nitrate could be used on each of the respective soils with profit. The smaller rate, as 100 to 200 pounds an acre, seemed to be the best when applied in April.

The fertilizer test conducted in Alabama by Williamson, Appleton, and Helm (19), and by Cauthen and Williamson (3) showed that a moderate rate, from 120 to 200 pounds to the acre, was the most economical, and that the proper time for making the application was either 6 weeks after planting time or when the corn had attained a height of 2½ feet.

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<sup>1</sup> These data are taken from a thesis presented at Purdue University in partial fulfillment of the requirements for the degree of Master of Science. Published with the approval of the director of the Purdue University Agricultural Experiment Station.

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This brief summary of the literature shows that nitrate of soda can be used profitably, but still there was some disagreement on the best time and rate of applying the material. Also, the results obtained were applicable only to the section where the tests were conducted, so it seemed desirable that more definite information on the use of nitrate should be obtained by further field experimentation. Thus, in the spring of 1927 this investigation was begun in order to obtain more specific information as to the value of nitrate of soda on certain Indiana soils. The soils chosen for this work included some of the more important soils in Indiana. For the most part, the locations were established on the outlying fields of the Purdue University Agricultural Experiment Station or nearby.

#### EXPERIMENTATION

Field tests were conducted on corn, wheat, and timothy to determine the influence of nitrate of soda when applied at different times and various rates upon the yield, quality, and protein content of these three crops.

The laboratory studies were made on composite samples taken from the respective plot treatments at harvest time. The  $\frac{1}{16}$ -acre plot (14 feet by 155.5 feet) was used for all tests.

#### *Corn experiments*

The general plan of plot treatment for corn, as given in table 1, provided for a basal application of 300 pounds of 0-12-12 fertilizer to the acre at planting time, either by the broadcast method or in the row with a Planet Jr. fertilizer drill, and the application of the nitrate later at similar stages of growth at each location. The June applications of nitrate were made when the corn was approximately 18 inches high, and the July applications were made when the corn had attained a height of 3.5 to 4 feet. These stages for side-dressing corn were also recommended by Moores and Roberts (13) and by Duggar and Williamson (5). The quality of corn was determined at harvest time upon a basis of maturity and moisture and then was divided into sound and unmarketable classes. The sound corn included all sizes of ears that were sound and solid, whereas the unmarketable corn included ears of all sizes that were soft, moldy, or chaffy.

Table 2 presents the yield obtained from all the fertilizer tests on corn from every location established during 1927 and 1928. Several of the locations included the average of 2 years, whereas the others are the new tests established in 1928. More tests were laid out than are shown in table 2, but since some of these were coöperative tests no data were obtained on account of failure on the part of the coöperators.

The yield data of all plots are on a comparable basis as far as the stands were concerned. The number of stalks on each plot were counted and the actual yields were then corrected to the average stand of the entire series.

The soil of the Wanatah test, which is described as a light colored sandy

TABLE 1  
*Plan of treatment for corn experiment*

PLOT NUMBER	TREATMENT AND RATE PER ACRE
1	300 pounds 0-12-12 broadcast
2	300 pounds 3-12-12 broadcast
3	300 pounds 0-12-12 50 pounds nitrate in July
4	300 pounds 0-12-12 100 pounds nitrate in July
5	300 pounds 0-12-12 broadcast
6	300 pounds 0-12-12, 50 pounds nitrate in June 50 pounds nitrate in July
7	300 pounds 0-12-12, 100 pounds nitrate in June 100 pounds nitrate in July
8	200 pounds 0-12-12 broadcast 100 pounds 0-12-12 in row
9	300 pounds 0-12-12 broadcast
10	200 pounds 0-12-12 broadcast 100 pounds 3-12-12 in row
11	100 pounds 0-12-12 in row
12	100 pounds 3-12-12 in row
13	200 pounds 0-12-12 broadcast, 100 pounds 0-12-12 and 50 pounds nitrate in row + 150 pounds nitrate when corn was 9 to 12 inches high
14	300 pounds 0-12-12 broadcast

TABLE 2  
*The effect of nitrate of soda on the yield of corn*  
Bushels per acre

PLOT NUMBER	VINCENNES 1927-28*	BEDFORD 1927-28	WANATAH 1927-28	WORTH- INGTON 1927	NORTH VERNON 1928	FARMLAND 1928	CULVER 1928	AVERAGE 1927-28
1	54.6	53.3	30.6	37.2	41.5	34.6	7.4	39.8
2	61.6	60.8	34.2	40.7	43.6	35.5	10.1	44.3
3	63.3	59.4	38.4	38.2	50.3	33.3	17.4	46.1
4	65.8	60.0	37.5	39.5	54.9	29.5	26.5	47.7
5	59.3	54.8	36.4	43.5	43.3	24.5	8.8	42.5
6	70.4	53.6	44.4	40.8	52.6	27.1	20.3	47.8
7	71.8	53.1	45.3	41.4	57.4	33.5	33.4	50.7
8	63.2	52.0	35.6	43.0	39.4	36.7	12.1	42.3
9	62.9	49.2	33.3	43.9	42.3	30.7	5.9	40.4
10	63.5	53.3	39.4	46.5	51.1	26.8	9.8	44.6
11	63.4	55.8	36.4	41.3	53.1	31.3	8.8	49.5
12	60.7	48.1	36.0	42.5	50.8	40.4	10.7	43.4
13	75.1	68.6	35.7	....	....	30.0	33.4	48.6
14	52.2	57.2	26.1	42.3	45.2	22.3	7.3	38.9

\* 1927-28 represents the average of the two years.



loam soil and somewhat acid, responded to nitrate fertilization in that all nitrate treated plots gave an increase, but some proved more economical than others. The data show that the divided applications, one-half in June and half in July, gave the best returns, whereas plot 6, with the 100-pound divided application, gave the greatest net profit per acre. The row applications, including approximately 50 pounds of nitrate at planting time, were not very significant, as these treatments gave contradictory results during the 2 years of the experiment. The increases obtained from plot 13 were hardly sufficient to equal the cost of the nitrate and cost of application.

The difference in quality was very noticeable in the 1928 yields. A large type of Reid's Yellow Dent was the variety grown this year, and as a consequence it was caught by the early September frost. The nitrate of soda treated plots had a greater proportion of sound corn than did the plots receiving no nitrate. However, all of the corn had a very high moisture content and the weight per bushel was calculated on a basis of 90 pounds per bushel.

Only the 1928 yields were obtained on the Farmland test, as the 1927 test was discarded because of the very poor stand. The soil of this test is classified as Crosby silt loam, a poorly drained, heavy upland soil. The quality of the corn was very uniform, all coming under the classification of sound corn. The yields of this test are rather small for nitrate application and hardly equal the cost of the fertilizer. The results of plot 12 are rather erratic and cannot be explained, as the other row applications of the small amount of nitrate at planting time did not give any such increase. This in part was attributed to soil variation, as the plot was rather outstanding all through the season.

The average yields of the Bedford test, located on Bedford silt loam in southern Indiana in the limestone area, show that the July applications were the best. Part of these slight increases for nitrate were attributed to the season rather than the nitrogen. The 1927 season was so wet and cool that the planting of the corn was delayed until a late date. The seasonal conditions were also adverse for proper development during the short growing season. Here again, the 50-pound application gave the highest net return, while plot 2, with approximately the equivalent of 60 pounds per acre, was next in profitability. Since the weather conditions for the 2-year period were contradictory, the nitrate responses were not outstanding.

The Vincennes test was located on a Gibson sandy loam soil in Southwestern Indiana. The data show that all treatments, irrespective of the amount, gave relatively good response to the nitrate fertilization. The only treatment that failed to give a response was the 3-12-12 applied in the row. However, plot 13, with the same treatment plus an additional amount of nitrate when the corn was over 6 inches high showed that plot 12 suffered from a deficiency of nitrogen later in the growing season. The best treatment was that on plot 13, which gave a 24.8-bushel increase. July seemed to be the best time for making the application on this light sandy soil, and the 50-pound rate gave the largest net profit for the amount invested.

The Culver test, located on Plainfield sand, gave very striking increases for the nitrate, but this was expected, as this soil is extremely low in fertility, as shown by the yields of the check plots. The large increases in yields were obtained from the July applications. Both gave about the same increase in proportion to the amount of nitrate applied. This increase of the July application was most effective, as the corn was at sufficient height to assimilate the nitrate in the most economical manner. The divided applications gave good increases, but were not so effective as half the rate when applied in July. The largest bushel increase, 26.3 bushels, was gained from plot 13, but when the net returns were computed this treatment returned about half the net profit produced by the July applications. Nevertheless, a good return was obtained on plot 13 for the amount invested in nitrate of soda.

TABLE 3  
*Average protein content of corn as influenced by nitrate of soda during 1927-28*

PLOT NUMBER	VINCENNES 1927-28	BEDFORD 1927-28	WANATAH 1927-28	NORTH VERNON 1927	FARMLAND 1928	CULVER 1928	AVERAGE 1927-28
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	7.8	7.8	9.3	6.4	9.2	6.1	8.1
2	7.8	7.4	9.4	6.7	9.2	6.3	8.0
3	8.2	7.6	9.7	7.0	8.4	7.4	8.4
4	8.6	8.1	9.8	7.6	8.6	7.1	8.4
5	7.8	7.8	9.3	6.7	8.9	6.7	8.1
6	8.2	7.9	9.5	7.2	9.1	6.5	8.3
7	8.6	8.5	10.0	8.1	9.6	7.5	8.9
8	8.1	8.2	9.5	7.2	8.6	6.3	8.4
9	7.7	8.0	9.4	6.6	9.6	6.2	8.2
10	7.8	8.1	9.7	7.0	9.2	6.3	8.1
11	8.0	7.8	9.2	7.3	10.0	6.9	8.4
12	7.8	7.6	9.3	7.3	10.2	6.9	8.3
13	8.1	7.6	8.7	...	10.6	7.3	8.6
14	8.0	8.3	9.3	7.7	10.0	6.6	8.5

The Clermont silt loam soil of the North Vernon test is a flat, poorly drained, whitish gray soil of rather low fertility, but is quite responsive to fertilizers when properly drained. The practice of side-dressing corn with nitrate proved very effective, as substantial increases in yields were obtained from all nitrate plots. The outstanding one was the 50-pound application in July which gave a 7.9-bushel increase. The other side-dressings of nitrate gave responses ranging from 10 to 16 bushels, but when compared in proportion to the amount of nitrate applied the 50-pound application gave the most economical returns.

The protein content of corn is summarized in table 3. One readily sees that the protein content is influenced by the soil type and local climatic conditions. The poor soil types as the Clermont silt loam or the Plainfield sand have a much lower percentage than the other soil series of the experiment. However, these local factors have not offset the influence of the nitrate. The data clearly

show that the heavy applications have increased the protein content more than the light rates. The 200-pound application in April gave an increase of approximately 0.8 per cent, whereas the 100-pound application gave an appreciably lower increase. Also, the July application had more effect than the early June application. In general, nitrate fertilization does exert some influence upon the protein content.

*Summary of corn experiments.* The results of the first 2 years of this experiment show that nitrate of soda can be used as a fertilizer on Indiana soils with a fair degree of profit. The 50-pound application applied in July gave an average increase of 5 bushels of corn an acre, and the 100-pound July application gave an average increase of 5.8 bushels. The 100-pound divided application yielded approximately the same, 5.9 bushels. The 200-pound application gave a 9.3 bushel increase.

The small amounts applied in the row at planting time did not prove practical, as this early stimulation caused the formation of too large a plant. This later caused the plant to suffer because of the lack of available nitrogen.

#### *Wheat experiments*

A series of 10 one-twentieth acre plots were used for the wheat experiments that were laid out on bulk wheat fields on various soils of the state. Special care was taken in the choice of these fields for the experiment so that no nitrate fertilizers had been applied on them except for a small amount at seeding time in the fall. The general type of treatment is as follows:

1. No nitrate
2. 50 pounds in April
3. 100 pounds in April
4. 200 pounds in April
5. No nitrate
6. 100 pounds in May
7. 50 pounds in April and 50 pounds in May
8. 100 pounds in April and 100 pounds in May
9. 150 pounds in April
10. No nitrate

The nitrate applications were made on wheat at similar growth stages. The April applications were made when the wheat was approximately 4 inches high, and the May applications were made when the wheat was 12 to 15 inches high.

The influence of nitrate of soda on wheat yields is shown in table 4, which includes all locations harvested during 1927 and 1928. The results show that the individual soils responded somewhat differently to the added nitrate, but on the whole the April applications were generally the best. Each test will be discussed separately.

The Vincennes test was located on Gibson silt loam soil. Increases in yields resulted for every treatment. However, the outstanding treatment both

years of the test was the 50-pound application in April, giving a 2.8-bushel increase, while the 100-pound application gave almost as good returns in proportion to the amount of nitrate, with a 5.0-bushel increase. The May applications seemed to be rather late for stimulating the yields in this southern section.

The average yields for the 2-year period on Bedford silt loam did not show very high increases. This in part may be attributed to the 1928 yields, as the stand for this year was thin as a result of severe winter injury. Nevertheless, the early nitrate application encouraged a thickening of the stand. The April treatment was thus sufficient to produce a higher yield than the May application. This test credits the 100-pound April treatment as the best for this soil with an average increase of 4.5 bushels.

TABLE 4  
*Average results of top-dressing wheat with nitrate of soda in 1927-28*  
Bushels per acre

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VINCENNES 1927-28	BEDFORD 1927-28	NORTH VERNON 1927-28	LAFAYETTE 1927-28	WANATAH 1927-28	CULVER 1927-28	CONNERSVILLE 1928	AVERAGE BUSHELS 1927-28
1	No nitrate	13.4	16.9	8.5	24.8	13.4	11.8	22.3	14.8
2	50 in April	16.7	17.0	10.3	27.6	21.5	14.6	24.7	17.2
3	100 in April	19.4	17.3	11.0	29.6	24.8	14.6	27.3	19.3
4	200 in April	22.1	21.3	13.2	29.9	28.5	21.3	25.3	23.1
5	No nitrate	15.5	13.3	9.8	24.1	19.1	10.2	19.3	13.6
6	100 in May	...	15.0	11.4	27.0	23.8	...	22.7	18.1
7	50 in April + 50 in May	18.3	16.1	14.0	30.0	23.5	...	31.3	19.9
8	100 in April + 100 in May	23.4	17.9	17.9	31.6	27.6	...	24.7	21.6
9*	150 in April	...	8.0	13.0	22.2	35.0	20.7	26.7	21.1
10	No nitrate	16.3	12.3	9.3	25.0	16.6	12.2	23.7	15.4

\* 1928 yields only.

The Clermont silt loam, the soil of the North Vernon test, was responsive to nitrate during the first 2 years of the investigation. This soil is poorly drained and as a consequence is rather late in warming up in the spring, so the natural nitrification is impeded during these early spring months. Thus, nitrate additions were very beneficial both in April and May. The 100-pound divided application, one-half in April and one-half in May, was the outstanding treatment of the test giving a 4.5-bushel increase. This was followed by plot 8 with a similar type of treatment except that a total of 200 pounds was applied. This resulted in an average increase of 6.5 bushels. The 100-pound application in May, 1928, was almost as profitable as the 100-pound divided application, but the average for the 2 years was materially reduced by the lateness of the 1927 application and which was applied close to heading time. As a

consequence, this was too late for stimulating yields. The April treatments were probably not all utilized by the small plants and in all probability some nitrate was lost in the drainage water.

The Lafayette test was laid out in 1927 on Brookston silt loam, a dark colored soil; the 1928 test was laid out on Crosby silt loam which was somewhat lighter in color and contained slightly less native fertility than the Brookston soil. Nevertheless, good increases were obtained for all nitrate treatments during both years. The increases on the Brookston silt loam were not as great as on Crosby, especially the 200-pound April application, which was too heavy for this soil and resulted in a poorer quality of wheat, as a result of lodging of the grain before maturity. Substantial increases were obtained from all the other treatments. The 50-pound application in April was the best in this test, giving a 3-bushel increase; the other nitrate applications were slightly lower in proportion to the amount of nitrate applied.

The response to nitrate of soda fertilization on Plainfield sand at the Culver location was very pronounced for all the different applications. This was expected on this soil, because of its low native fertility, being especially lacking in nitrogen. Since the May applications of this test were spoiled in 1928, no average yields were obtained. However, the 1927 yields showed that additional nitrate applied on this open, sandy soil was beneficial, giving a much greater response when applied either as a divided application or all in May. These data showed that all rates and all times of application gave good returns on the investment. The 50-pound application in April was exceptionally effective, yielding a 2.2-bushel increase over the untreated plot.

The Connersville test was established on Russell silt loam in 1928. The yields for this season gave the 100-pound divided application the advantage, resulting in an increase of 10.2 bushels, whereas 50- and 100-pound applications in April were very good but were slightly lower in their net increases for the amount of nitrate applied. However, these data show that nitrate of soda can be used very effectively up to 100 pounds an acre.

The quality of the wheat was determined on the laboratory studies based on protein content and test weight per bushel. The test weight was measured by the official method, and the official Kjeldahl method was used for the protein determinations. The results for these studies, as given in tables 5 and 6, are based only on the 1928 yields. These data are somewhat variable, being slightly influenced by the local climatic conditions and the soil type. However, the nitrate of soda additions have caused some outstanding differences, mainly, that the April applications gave a lower percentage of protein than the corresponding May applications. There was a direct proportional increase for the April additions in favor of the increased amount of nitrate. The same is true with respect to rates of the May applications. However, the May applications are the highest as they were applied closest to the time of maturity. These results corroborated the studies of Davidson (4), Gericke (6), Shaw (16), and Neidig and Snyder (14) on the protein content of grain.

The test weight per bushel shows slight fluctuations with respect to the individual soil series. On the average, the April applications gave a slight decrease, whereas the May applications gave an increase. In general, the test weight per bushel was increased in cases where there was an abundance of the

TABLE 5

*The effect of various applications of nitrate of soda on the test weight per bushel of wheat grain in 1928*

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VIN- CENNES	BED- FORD	NORTH VERNON	CON- NERS- VILLE	LAFAY- ETTE	CULVER	WAN- ATAH	AVER- AGE
		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1	No nitrate	59.3	55.4	58.9	57.0	60.2	57.9	57.4	58.0
2	50 in April	59.0	54.7	58.5	57.0	60.1	58.7	57.4	57.9
3	100 in April	58.4	54.7	58.9	57.0	59.8	58.4	57.4	57.8
4	200 in April	57.7	55.7	58.7	57.0	59.8	59.4	58.5	58.1
5	No nitrate	57.5	55.9	59.0	55.0	60.3	58.1	57.5	57.6
6	100 in May	...	54.2	58.0	56.0	61.1	...	58.5	57.7
7	50 in April + 50 in May	57.6	54.6	58.6	56.5	60.7	...	58.2	57.7
8	100 in April + 100 in May	57.6	54.1	55.6	55.0	61.1	...	58.0	57.0
9	150 in April	...	55.0	56.0	56.0	61.4	58.0	58.7	57.5
10	No nitrate	59.0	55.3	56.0	56.0	60.4	58.0	57.8	57.5

TABLE 6

*Percentage of protein in wheat as influenced by various applications of nitrate of soda in 1928*

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VIN- CENNES	BED- FORD	NORTH VERNON	CON- NERS- VILLE	LAFAY- ETTE	CULVER	WAN- ATAH	AVER- AGE
1	No nitrate	9.7	9.0	9.1	9.3	9.1	9.9	9.6	9.4
2	50 in April	10.5	10.5	9.0	9.8	9.3	9.5	9.6	9.7
3	100 in April	10.6	10.7	9.0	9.4	9.5	9.6	9.6	9.8
4	200 in April	11.2	10.5	9.3	9.5	9.6	11.0	10.6	10.2
5	No nitrate	9.6	9.6	9.4	9.5	9.2	10.0	9.4	9.5
6	100 in May	...	11.2	9.0	9.5	10.8	...	10.4	10.2
7	50 in April + 50 in May	11.1	10.9	9.0	9.6	9.4	...	10.1	10.0
8	100 in April + 100 in May	11.2	11.2	9.3	9.6	10.5	...	10.8	10.4
9	150 in April	...	11.1	9.2	9.4	10.5	9.5	10.7	10.1
10	No nitrate	9.7	10.0	8.9	9.4	9.7	9.9	9.1	9.5

other fertilizing elements present, and the reverse condition resulted where there was a shortage of essential elements.

In considering the average return from treating these Indiana soils with nitrate, it is evident that the April top-dressings were the most profitable, especially the smaller rate of application. The 50-pound application gave an

average increase of 2.7 bushels, whereas the 100-pound application gave a 5.0-bushel increase, which was slightly lower in proportion to the amount of nitrate. The 100-pound application, in which 50 pounds was applied in April and 50 pounds in May, gave the largest acre increase, 5.5 bushels. However, when the extra cost of applying the material in two installments is considered, the net profit would be reduced somewhat, and in all probability the single April application would be the most practical for average farm conditions. The 200-pound rate in April gave 7.8 bushels increase, whereas the 200-pound divided application on plot 8 gave only 6.7 bushels.

### *Timothy experiments*

The field tests for timothy were conducted on  $\frac{1}{4}$ -acre plots but were laid out in duplicate at each location in order to facilitate weighing at harvest time. Thus, all yields recorded are an average of duplicate tests. The general plan for the nitrate experiment is given in the following:

#### *Plan of timothy experiment*

1. No fertilizer
2. 100 pounds 0-12-12
3. 100 pounds 0-12-12 plus 100 pounds of nitrate in April
4. No fertilizer
5. 100 pounds 0-12-12 plus 200 pounds of nitrate
6. 100 pounds 0-12-12 plus 300 pounds of nitrate
7. No fertilizer

The average results of applying nitrate of soda to timothy are summarized in table 7 for all locations harvested during 1927 and 1928. The top-dressings were made early in the spring usually when the timothy had made a fair growth and was 4 to 6 inches high.

The North Vernon test was located on Clermont silt loam on adjacent farms for the 2-year period. The meadow both years was a 2-year-old one. The timothy was allowed to dry thoroughly before the weighings were made. The yield data show that all rates of nitrate applied were effective in that all yields were in proportion to the amount of the application. However, the 200-pound rate was the most effective, giving an increase of 1,565 pounds of hay over the check plot, whereas the 300-pound application gave an increase of 2,227 pounds of hay. The differences in maturity were very noticeable, as the crop receiving the heavy nitrate application was slightly earlier, whereas that receiving the lighter application and that from untreated plots were decidedly later. However, the heavy nitrate plots had a tendency to produce a slightly coarser hay.

The Columbus test was laid out on Clermont silt loam in Bartholomew County. This was a 2-year-old meadow. Here again, the response to nitrate was restricted, because of the extremely low fertility of this "slash land" soil. The 100-pound application was the best, producing a 1,064-pound increase,

whereas only 1,343 and 1,930 pounds of hay were the increases received from the 200-pound and 300-pound applications respectively. The effects of the nitrate were shown by a thickening of the stand, restriction of the weeds, and a reduction of clover.

The Monticello test was conducted in White County on light, sandy soil. The beneficial effects of the nitrate were observed soon after the initial application, and at harvest time the nitrated plots were 10 inches taller than the untreated plots. The actual increases for the 2-year period are proportional to the increasing rate of application. However, the 200-pound application was the most economical, giving a 2,173-pound increase, whereas the 300-pound application yielded a 2,424-pound increase. Here again, the 300-pound application was too heavy. This was shown by the coarse woody condition of the stems and a browning of the leaves.

TABLE 7  
*Average yields of timothy top-dressed with nitrate of soda at each location*

PLOT NUMBER	TREATMENT PER ACRE	NORTH VERNON 1927-28	MONTICELLO 1927-28	COLUMBUS 1927	WORTHINGTON 1927	CULVER 1927	CRAWFORDSVILLE 1928	AVERAGE FOR ALL TESTS 1927-28
		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1	No fertilizer	2,060	2,035	1,040	2,200	1,480	1,210	1,820
2	100 pounds 0-12-12	2,350	2,040	1,360	3,040	1,640	1,210	2,031
3	100 pounds 0-12-12	2,230	3,080	2,540	4,520	2,420	2,178	2,992
	100 pounds nitrate							
4	No fertilizer	2,205	2,280	1,380	3,260	1,280	1,210	2,015
5	100 pounds 0-12-12	3,980	4,310	3,000	6,360	3,640	2,672	3,985
	200 pounds nitrate							
6	100 pounds 0-12-12	4,420	4,560	3,660	6,240	4,560	3,267	4,336
	300 pounds nitrate							
7	No fertilizer	1,975	2,280	1,600	3,900	1,490	1,090	1,994

The Worthing test was established in 1927 in Greene county on a heavy silt loam. This was the first year's crop for this meadow. All through the season the nitrate plots were superior and the increases obtained from this test show that nitrate can be used with profit up to 200 pounds an acre. When nitrate was applied much above this rate, the quality was reduced and the timothy lodged rather badly. The 100-pound application produced a 1,134-pound increase, and the 200-pound application gave an increase of 2,380 pounds.

The Culver test was laid out on sandy loam soil in Marshall County on a new timothy meadow. The nitrate of soda was very effective on this field, as increased yields were obtained for each additional 100 pounds. The 300-pound application gave the largest increase in proportion to the amount of nitrate applied. However, good increases were received from the other nitrate applications. The 100-pound application gave an increase of 846 pounds of hay, and the 200-pound application of nitrate produced a 2,060-pound increase.



The Crawfordsville test was located on Miami silt loam on a 2-year-old meadow. Nitrate of soda was effective in producing increases, but the increases were not so outstanding as on the other soil types used in this experiment. The 100-pound rate was most effective in proportion to the amount applied, giving an increase of 968 pounds an acre. The higher rates of application gave very little for the additional nitrate.

*Summary of timothy experiments.* The data obtained from the experiments in this project show that nitrate of soda gave increases in hay yields of 927, 1,876, and 2,210 pounds for the 100, 200, and 300-pound applications respectively. Thus, for average Indiana conditions the 200-pound application proved to be the most profitable, giving the best proportionate increase as well as the best quality of hay.

The results show that nitrate of soda applied to timothy meadows in early spring was responsible for stimulating growth, increasing the stand, and restricting the growth of weeds.

#### SUMMARY

The favorable results obtained from the use of nitrate of soda during the 2 years of this investigation may be in part attributed to seasonal influence. In both years cool weather was prevalent during the early part of the growing season, and caused a general nitrate deficiency. Thus, the nitrate of soda was very beneficial in increasing yields. However, these results may be altered by the local variation in temperature or rainfall.

The average crop increases produced by the nitrate of soda applications on corn, during the 2 years, were barely sufficient to pay the cost of the nitrate. The 50-pound application in July was the only application to give a net profit.

In general, the small amounts of nitrate applied in the row at planting time on corn gave a stimulating effect at first, but caused the crop to suffer from lack of available nitrogen later in the season, thus causing a decrease in yield.

The average of the 2 years' data on applying nitrate of soda to wheat shows that the smaller rates of application, 50 or 100 pounds, were the most profitable, and that the April applications proved to be the best.

The results obtained from top-dressing timothy meadows with nitrate of soda showed that moderate amounts were beneficial in increasing yields, in stimulating growth, and in limiting the growth of weeds.

The protein analyses of the three field crops for the 2 years show that the percentage of protein is raised to a slightly higher degree by late application as well as by a heavier application of nitrate of soda.

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# THE LAWS OF SOIL COLLOIDAL BEHAVIOR: X. EXCHANGE NEUTRALITY AND COMBINING CAPACITY<sup>1</sup>

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The soil colloidal complex represents salts of weak acids (silicic, humic, etc.) and bases (chiefly of aluminum and iron). Because of their weakness these acids and bases neutralize each other only partially, leaving an acid and a basic residue too weak for a mutual interaction. These compounds react therefore amphotERICALLY at higher pH with bases, and at low pH with acids. Their isoelectric point, that is, the point at which the acid and basic residues are equally ionized, depends upon the relative strength of the acid and basic radicals. The soil complex is, with few exceptions, isoelectric on the acid side of neutrality. The acid residue is therefore usually stronger than the basic.

Since the weak acids and bases are mostly themselves highly insoluble compounds it is clear that the composition of the complex can vary within the widest limits. Thus we may have compounds of the following types in which A and B represent trivalent acid and basic groups respectively:

- |     |   |   |
|-----|---|---|
| (A) | $\begin{array}{c} \text{H}_2 \text{ A} \backslash \\ \text{B OH} \\ \text{H}_2 \text{ A} / \end{array}$ | cation combining power high;<br>anion combining power low;<br>isoelectric pH low;<br>ultimate pH low.       |
| (B) | $\text{H}_2 \text{ A} - \text{B(OH)}_2$   | cation and anion combining power intermediate;<br>isoelectric pH intermediate;<br>ultimate pH intermediate. |
| (C) | $\begin{array}{c} \text{B(OH)}_2 \\ / \text{ H A} \\ \backslash \text{ B(OH)}_2 \end{array}$            | cation combining power low;<br>anion combining power high;<br>isoelectric pH high;<br>ultimate pH high.     |

These formulas are merely given to represent different steps in the ratio between the acid and basic radicals. It is obvious that in a colloidal aggregate containing millions of molecules any composition is possible. We thus have every transition in the composition of soil colloids from those which are high in silica to those having a high sesquioxide content.

Hand in hand with this gradation in composition we find a gradation in the

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amphoteric behavior and reactivity of the colloids as indicated by the foregoing scheme. The power to adsorb and exchange cations, which increases with increasing pH, is proportional to the strength (dissociation) and quantity (equivalence) of the acid residue whereas the power to adsorb and exchange anions, which increases with decreasing pH, is proportional to the strength and quantity of the basic residue. The pH of exchange neutrality (3, VI), that is, the point at which the free ampholytoid combines with an equal number of the cations and anions in a particular salt solution in exchange for H and OH ions, is therefore, like the isoelectric point, dependent upon the composition ratio or, more correctly, upon the ratio of the activity of the acid residue to that of the basic residue.

Since most of the properties of soil colloids are directly or indirectly functions of this dual character to react with acids and bases and with anions and cations it is obviously of the utmost importance to find a simple procedure whereby their reaction capacity and their point of exchange neutrality may be determined. This may be found quite simply by determining the neutralization curve for the electro dialyzed soil in a series of neutral salt solutions to which free acid on the one side and free base on the other have been added as previously described (3, VI). In the following pages we shall present the results of this method applied to various soils and other materials. For a theoretical discussion of the neutralization of amphoteric colloids the reader is referred to the preceding section of this series (3, IX).

In determining the power of a colloid to bind acid or base it is, of course, as imperative that the colloid be in the free acid-base condition as it is that an ordinary acid or base be in the free, uncombined condition when such a determination is to be made. This is most easily accomplished in the case of soils by electro dialysis. The soil complex is thereby converted into the free acid-base-ampholytoid.

The pH of the electro dialyzed soil represents its ultimate value and expresses the relative strength of the acid and basic residue. The ultimate pH is low in soils whose complex is high in silicic and humic acids, whereas the ultimate pH is high in laterites and other soils high in sesquioxides.

When an electro dialyzed soil is added to a neutral salt solution an exchange acidity is usually developed, since most soils possess a stronger acid than basic residue. (Aluminum hydroxide and some extreme soil types isoelectric above pH 7.0 yield an exchange alkalinity.) The pH developed by the exchange reaction between the electro dialysed soil and the neutral salt solution depends (a) upon the amphoteric nature of the colloidal complex of the soil and (b) upon the nature of the ions of the salt. Since the neutral salt solution possesses practically no buffer capacity the pH at equilibrium with the electro dialyzed soil will very nearly represent the point of exchange neutrality, that is, the pH at which the anions and cations of the salt are adsorbed in equal numbers. The  $\text{SO}_4$  ions displace the OH ions more energetically than do the Cl ions (3, III). This we ascribe to a higher association, i.e., a lower dissociation, of the  $\text{SO}_4$  ions.

The pH of exchange neutrality is therefore higher in a  $\text{Na}_2\text{SO}_4$  solution than in a  $\text{NaCl}$  solution. The Ca ions displace the H ions more strongly than do the Na ions. The pH of exchange neutrality is therefore lower in  $\text{CaCl}_2$  than in  $\text{NaCl}$  (3, V). The point of exchange neutrality, therefore, does not necessarily coincide with the isoelectric point. The former point represents the pH at which the colloid *combines* with an equal number of anions and cations of the salt, whereas the latter point is defined as the pH at which the colloid *dissociates* an equal number of anions and cations.

In the following experiments a normal solution of  $\text{Na}_2\text{SO}_4$  was employed. The advantage of using a neutral salt solution in determining the neutralization curve of a soil is twofold. In the first place it yields a significant point of origin, or zero point, as represented by the pH of exchange neutrality. In the second place the presence of a large amount of neutral salt greatly hastens the displacement, which otherwise proceeds very slowly upon the additions of small increments of acid or base. The equilibrium position is, however, displaced by the presence of the salt, which causes more of the anions and cations to combine at a given pH.

#### EXPERIMENTAL PROCEDURE

A series of stock solutions of  $N$   $\text{Na}_2\text{SO}_4$  were prepared by adding increasing quantities of  $\text{H}_2\text{SO}_4$  on the one side and  $\text{NaOH}$  on the other. The dotted S curves in the figures marked " $\text{Na}_2\text{SO}_4$  solution" show the relationship between the pH (ordinates) and the milliequivalents of acid or base contained in 25 cc. of solution (abscissa).

One gram (except where otherwise stated) of the electro-dialyzed soils or other materials was shaken with 25 cc. of the various solutions and let stand over night. The pH was then determined colorimetrically in the clear supernatant liquid and a curve plotted for each material to the same scale as the curve representing the original salt solutions.

Every material which reacts amphotERICALLY will yield a curve which intersects the salt solution curve. The point of intersection marks the pH of exchange neutrality, whereas the horizontal distances between the two curves yield, at any one pH when projected on the abscissas, the *net* combining power in milliequivalents per gram for acids (to the left) and bases (to the right).

#### THE REACTION OF DIFFERENT SOIL TYPES

We shall first present the results obtained from three soils which represent extreme variations in composition; namely, the Sharkey, the Sassafras, and the Nipe soils. The colloids of these soils have already been discussed in respect to many of their properties. The ratio of silica to sesquioxide and the cation exchange capacity at pH 7.0 of these colloids will serve to account for the reaction of the soils although the colloids were extracted from different samples. The figures are as follows:

COLLOID	SHARKEY	SASSAFRAS	NIPE
SiO <sub>2</sub> .....	3.18	1.89	0.31
R <sub>2</sub> O <sub>3</sub> .....			
Exchange capacity, milliequiv/gram .....	0.80	0.33	0.04

Figure 28 shows the pH of exchange neutrality in Na<sub>2</sub>SO<sub>4</sub> of the three (whole) soils as well as their reaction capacity, or more specifically, their power to bind H<sub>2</sub>SO<sub>4</sub> and NaOH in the presence of *N* Na<sub>2</sub>SO<sub>4</sub>.

The *pH of exchange neutrality* in the Na<sub>2</sub>SO<sub>4</sub> solution, i.e., the points of intersection, are found to be: Sharkey, 3.7; Sassafras, 4.7; Nipe, 6.1.

These values for the Sharkey and the Sassafras soils are somewhat higher than those previously reported for the exchange neutrality of their colloids in NaCl (3, VI). This is partly because the SO<sub>4</sub> ions are more strongly adsorbed than the Cl ions. The adsorption of the SO<sub>4</sub> ions will therefore balance the cation adsorption at a higher pH. A different salt effect on the indicator is another factor.

The *ultimate pH*, that is, the pH of the electrodialyzed soils, was determined in a ratio of soil to water of 1:2 and was as follows: Sharkey, 3.3; Sassafras, 4.45; Nipe, 6.0.

The ultimate pH at the dilution investigated was therefore somewhat lower than the pH of exchange neutrality in *N* Na<sub>2</sub>SO<sub>4</sub> solution (dilution 1:25). This indicates a strong adsorption of the SO<sub>4</sub> ion but the, here one-sided, salt effect renders a comparison uncertain. These two points are, as already pointed out (3, VI), related to, but not identical with, *the isoelectric point*. All three of these points are governed by the relative strength of the acid and basic residues. The stronger the acid and the weaker therefore the basic residue, the lower will be the pH of either of these points or vice versa. These points might be called *the amphoteric points* of the colloidal complex. (The ultimate pH is a value which is not alone confined to amphoteric colloids, for it is obviously also associated with colloids which are strictly acidic or basic in behavior.)

The amphoteric points are therefore intensity factors and express the quality of the soil complex as distinguished from the quantitative factors and measurements which have in the past chiefly attracted the attention of soil chemists. How useful and how essential a study of these qualitative characteristics of a soil are for an understanding of the soil processes will be brought out later. We shall now turn our attention to the quantitative aspects of the soils as shown in figure 28.

The *cation exchange capacities* at pH 7.0 as determined by leaching the soils with *N* barium acetate, washing and displacing the combined Ba with NH<sub>4</sub>Cl, were, in milliequivalents per gram, as follows: Sharkey, 0.41; Sassafras, 0.06 Nipe, 0.04.

If we now compare these values with the quantities of NaOH with which the soils have combined at a pH of 7.0 as shown in figure 28 by the points at which

the neutralization curves intersect the neutrality line, we note an almost perfect agreement in the case of the Nipe and Sassafras soils whereas the Sharkey shows a somewhat lower combining power, or about 0.35 m.e. NaOH as compared to 0.41 m.e. exchangeable Ba. Now it must be remembered that both the nature of the cations and the conditions of the experiments were different in the two cases. The two series of values should therefore not be expected to agree except in a general way. One method is as justifiable as the other for a determination of the "base exchange capacity" of a soil. Neither method corresponds to the equilibrium conditions in the field.

#### THE "TOTAL" COMBINING CAPACITY

The neutralization curves of the electrolyzed soils have not been carried beyond a certain point on the alkaline and acid sides because it is quite obvious that such a procedure would not yield the combining power of the soil complex as it originally existed. The soil complex is very unstable and undergoes an extensive hydrolysis and dissolution at high and low pH, culminating in silicates and aluminates on the one side and salts of aluminum and iron on the other.

The "total" combining power or exchange capacity of the colloidal complex is therefore a meaningless term. Furthermore certain soil complexes contain acid radicals too weak to come into play within the ordinary range of soil reactions which at higher pH bind large quantities of bases. Thus laterites having a high content of the weakly acidic, amphoteric alumina possess an extremely low exchange capacity at pH 7.0 whereas at high pH these soils may exceed, in their base binding power, other soils which possess a very much higher exchange capacity at the neutral point. The exchange capacity at pH 7.0 bears therefore no simple relationship to any "total" combining power. The degree of saturation based on any such total might be very misleading.

#### THE COLLOID CONTENT

The quantity of base adsorbed by a soil at any pH depends upon the combining capacity of the colloid and upon the quantity of the colloid in the soil. The combining capacity of the colloidal complex for bases depends upon the number of equivalents in the acid residue of sufficient strength to become neutralized at the pH in question. For a given soil the quantity of base adsorbed at any pH is proportional to the percentage of colloid present. The point of exchange neutrality, which is a qualitative expression, is independent of the amount of colloid present, obviously within certain limits of concentration. This is expressed in figure 29, which shows the neutralization curves of the Sassafras soil and colloid.

From the quantities of base adsorbed by the two materials at pH 7.0 (or any other pH above the point of exchange neutrality) we find that the colloidal fraction makes up about 31 per cent of the soil. The fact that the point of exchange neutrality is unaffected by the change in the colloid concentration is extremely important, since this constancy permits a characterization of the



soil complex without reference to its quantity, which is practically impossible to determine directly, i.e., by extraction.

#### EXCHANGE EQUIVALENTS

Figure 30 gives the neutralization curves of the three soils when taken in the proportion of their base combining equivalents at pH 7.0. The proportions were calculated from figure 28, which gives the combining power per gram soil under the same conditions. Eight grams of the Nipe and five of the Sassafras soil should be approximately equivalent to one gram of the Sharkey soil in respect to their power to neutralize NaOH at pH 7.0. It must be emphasized that these weights are equivalent only at the neutral point for which they are calculated.

It will be noted that the curves all intersect at a pH slightly above 7.0. The exactness of this point is of little significance for our present study. The deviation may be due to errors in the interpolation, or it may be a concentration effect. It may also be due to  $\text{CO}_2$  which would give the solution a certain buffer capacity. This would make the combining power of a soil appear greater than its true value and would affect soils of low combining power, such as the Nipe, much more than soils of the Sharkey type. If so, more than eight parts of the Nipe should have been taken.

The significant thing about the curves in figure 30 is their form as well as their intersection. The pH of exchange neutrality, i.e., the points of intersection with the  $\text{Na}_2\text{SO}_4$  solution curve, being unaffected, the slope of the Sassafras and especially that of the Nipe soil is greatly flattened. The buffer capacity per soil equivalent (not per gram soil) is greatest by far in the case of the Nipe soil, which adsorbs the base within the pH range of about 6.1 to 7.2 whereas the Sassafras covers a range from 4.6 to 7.2 and the Sharkey a range from 3.8 to 7.2 in adsorbing the same amount of base.

The fact that the curves originate at different points on the ordinate axis and the fact that they all intersect make it very obvious that the exchange equivalent weights of different soils may be very different in their relative magnitudes at different pH values. Thus the ratio 8 Nipe, 5 Sassafras, and 1 Sharkey holds for pH 7.0 only. At a higher pH the ratio will be narrower, whereas at a low pH it will be wider. At a pH well below 6.0 where the Nipe combines with and exchanges practically no cations this ratio approaches infinity. The reason for this difference is, as already stated, the differences in the strength and magnitude of the acid residue. The Nipe laterite may possess a very large acid residue but it is too weak to become engaged except at higher pH. The sesquioxides react as acids only at a high pH. The Nipe contains very little acid residue active below pH 7, such as silicic and humic acids. Titanium oxide is isoelectric at about pH 4.8 and binds a considerable amount of base at 7 but in most cases  $\text{TiO}_2$  makes up only from 0.5 to 1 per cent of the soil colloid complex. (The amphoteric titanium series are now being studied together with similar series of other elements in the periodic system. The results will be published shortly.)

## SATURATION, ALUMINUM SOLUBILITY, AND PLANT INJURY IN RELATION TO THE ISOELECTRIC POINT

The recognition of the amphoteric nature of the soil complex will prove very useful to soil chemistry. It will enable us to account for many phenomena which the one-sided conception of the soil complex as an acidoid could not do. Thus the "influence" of the anions upon the displacement of H ions by the neutral salt cations is easily accounted for when we know that the anions themselves enter into combination with the complex in increasing numbers as the pH is lowered, all depending upon the nature of the ions and upon the strength of the basic residue. Again, it will be readily understood why there can be no simple relationship between the pH and the degree of base saturation as would be expected if the soil complex represented a single, definite "soil acid."

Another phenomenon which has a bearing on the point of injury to plant growth may here be mentioned. Pierre (6) has made the important observation that the higher the percentage base saturation at a given pH the lower is the aluminum content in the solution. The obvious explanation of this is that the higher the base saturation at a given (low) pH the lower is the isoelectric point of the complex. This means a weak and less reactive basic residue, which requires a higher H-ion concentration for its ionization and solution, by hydrolytic cleavage, into mobile aluminum ions (single or complex). A low base saturation, at the same given pH, means, on the other hand, a high isoelectric point, a stronger and more active basic residue, and therefore a greater ionization and mobilization of aluminum.

The relationship is clearly shown by the three soils here studied. When the electrodialyzed soils (zero saturation) are treated with a neutral salt the aluminum in solution decreases in the following order:

Sharkey > Sassafras > Nipe

The exchange acidity is so much greater in the Sharkey that, although the complex of this soil not only contains less aluminum but holds it in a less active condition due to the strong acid residue [at a given pH the Sharkey colloid adsorbs by far the smallest quantities of neutral salt anions (3, VI)], more aluminum passes into solution from this soil than from the other two whose exchange acidity is much lower (comp. fig. 28). But if the pH of the Sassafras and the Nipe be brought down to that of the Sharkey by the addition of acid the order of the aluminum and iron solubility reverses itself and becomes

Nipe > Sassafras > Sharkey

exactly as the theory demands.

It is quite obvious that any attempt to relate the pH to aluminum solubility and plant injury must meet with failure until proper account is taken of the amphoteric nature of the soil studied. At a given low pH the Nipe soil would undoubtedly be the most toxic to plants, as it would then be on the acid and

electropositive side of its isoelectric point and would therefore yield a greater concentration of aluminum. But it must not be forgotten that the Nipe soil (laterite) would most strongly resist a lowering of the pH. Except under abnormal conditions this soil would always maintain a higher pH in the unsaturated condition. In fact, the ultimate pH of this type of soils is often so high that it is doubtful whether aluminum would ever, under normal conditions, become soluble to the extent of causing injury to plants. Manganese appears to be a greater source of danger in these soils, but this question will be dealt with later.

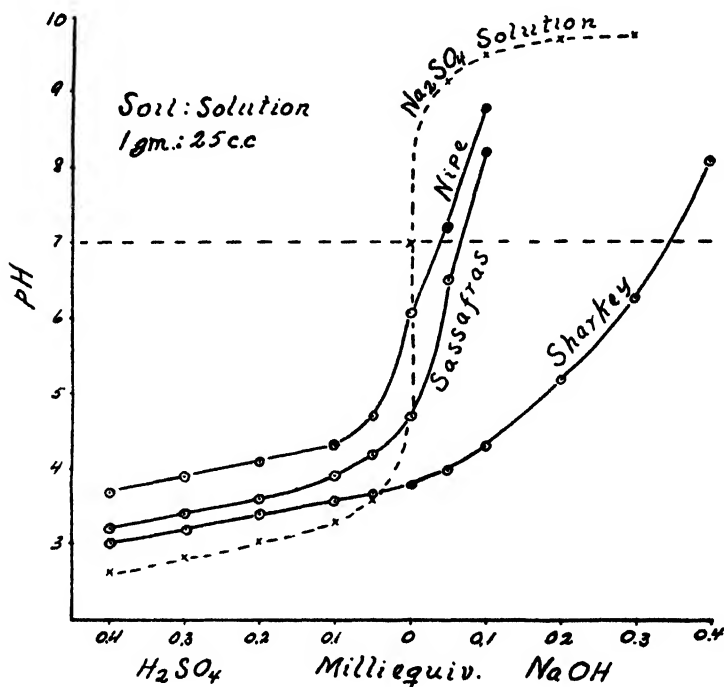


FIG. 28. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY OF THREE DIFFERENT ELECTRODIALYZED SOILS TO BIND ACID AND BASE

#### THE AMPHOTERIC REACTIONS OF PODZOLIZED SOILS

Very briefly stated, a podzolized soil is characterized by the following morphological and chemical differences (1):

The *A<sub>0</sub>* layer, or the uppermost layer, consisting of organic matter representing every stage of transformation. The humic material of this layer is usually very acid.

The *A<sub>1</sub>* horizon is the mineral horizon immediately below the *A<sub>0</sub>* layer. It contains a certain amount of humic materials and has a low pH.

The *A<sub>2</sub>* horizon when well developed is recognized by its ash-gray or bleached appearance. Its pH is then also low. The colloidal content of this horizon is relatively low. The mineral A horizons represent the zone of eluviation. From this zone certain materials, notably the

sesquioxides but also silicic and humic acids as well as strong bases, move downward in the dissolved or highly dispersed condition, only to be precipitated and accumulate at a greater depth.

The *B horizon* represents the horizon of accumulation or illuviation and is recognized by a greater compactness and a brownish color. The pH in this horizon is markedly higher than that of the preceding horizons. Its colloidal content is also relatively high.

The *C horizon* is represented by the underlying parent material. The pH in this horizon is usually the highest.

These changes within the podzolized soil body must primarily be ascribed to the prevailing pH in the various horizons. The pH is in turn conditioned by

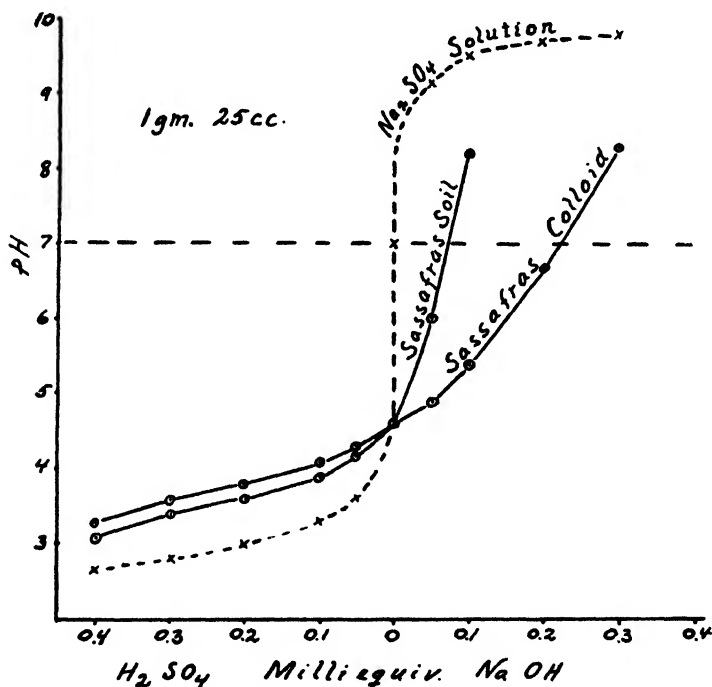
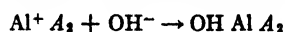


FIG. 29. THE pH OF EXCHANGE NEUTRALITY AND COMBINING CAPACITY OF THE ELECTRO-DIALYZED SASSAFRAS SOIL AND COLLOID

the climate and vegetation, which give rise to the accumulation of the sour, humic layer. A low temperature and a heavy rainfall together with a vegetation having a low ash content favor the formation of such a layer. The characteristic bleached podzol horizon is the result of an acid hydrolysis of the colloidal complex which follows an extensive leaching and unsaturation of the complex. This acid hydrolysis leads to an ionization of the basic sesquioxide constituent of the complex. Iron and aluminum move downward, chiefly in the form of ionic complexes, in union with organic (humic), silicic, and other acids. Aluminum and iron in "solution" at a pH of about 4.0 cannot exist as  $Al^{+++}$  and  $Fe^{+++}$  ions. Combinations like  $Al^{+}OH A$ ,  $Al^{+}A_2$ , etc. (where  $A$  = acid

anion) are certain to exist. These ionic complexes are precipitated in the B horizon where the pH is higher and the complex therefore more nearly isoelectric. The precipitation may be visualized as follows:



On the basis of the theory of isoelectric weathering, as outlined in the preceding part (3, IX) of this series, the composition of the amphoteric complex must be governed by the prevailing pH in such a way that the higher the pH the greater must be the basic, in this case, the sesquioxide proportion, in the complex; that is, the acidoid/basoid ratio must be low. A low pH, on the other hand, must lead to the formation of a complex in which this ratio is high. This is because the isoelectric pH, i.e., the pH of maximum stability, increases with the basoid or sesquioxide content and vice versa (3, III).

If this were so, then the colloidal complex left behind in the very acid A horizon should possess a high silica/sesquioxide ratio (acidoid/basoid ratio in general) and should yield relatively low amphoteric points. That is, the isoelectric pH, the pH of exchange neutrality and the ultimate pH should all be low. Such a complex can alone be stable in this horizon. The isoelectric point of the original complex (before podzolization) would be too high for the new, more acid, environment. By losing some of its sesquioxide component by the acid hydrolysis the complex automatically adjusts itself into a condition of stability.

The colloidal complex accumulating and built up in the B horizon where the prevailing pH is higher must, to be stable, assume a relatively lower acidoid/basoid ratio (since humic acids partly enter in place of silicic acid in this complex it is more rational to employ the general term for this ratio). The isoelectric point, the pH of exchange neutrality, and the ultimate pH must therefore be correspondingly higher in the B horizon as compared to the same values in the A horizons. The following investigation will show how well the theory fits the facts.

We selected for our study a sandy soil of the Lakewood series in the vicinity of Cedar Bridge, New Jersey. How well the morphological characteristics of the podzol profile have developed in this soil is shown in plate 2.

The area from which this profile was taken supported a forest growth predominantly oak with some pine mixed in, the average age being estimated at about 25 years. The shrubby and herbaceous ground cover was mostly ericaceous. There was ample evidence of recurring forest fires, and the general appearance of the stand indicated poor growth.

Table 95 shows the percentage of carbon and the pH of the original soil in the various horizons.<sup>2</sup> The table also gives the percentage of the major constituents and the silica/sesquioxide ratio of the extracted colloids. The latter were extracted by the supercentrifuge after dispersion in a slightly alkaline solution of  $\text{Na}_2\text{CO}_3$ .

<sup>2</sup> For the information contained in the table we are indebted to Dr. C. Watson who has made a separate study of this soil.

It will be seen that the pH increases downward from a value of 4.0 in the A<sub>1</sub> to 5.15 in the C horizon. The low pH in the A horizons has led to an extensive acid hydrolysis of the complex as indicated by the remarkably high silica/ses-

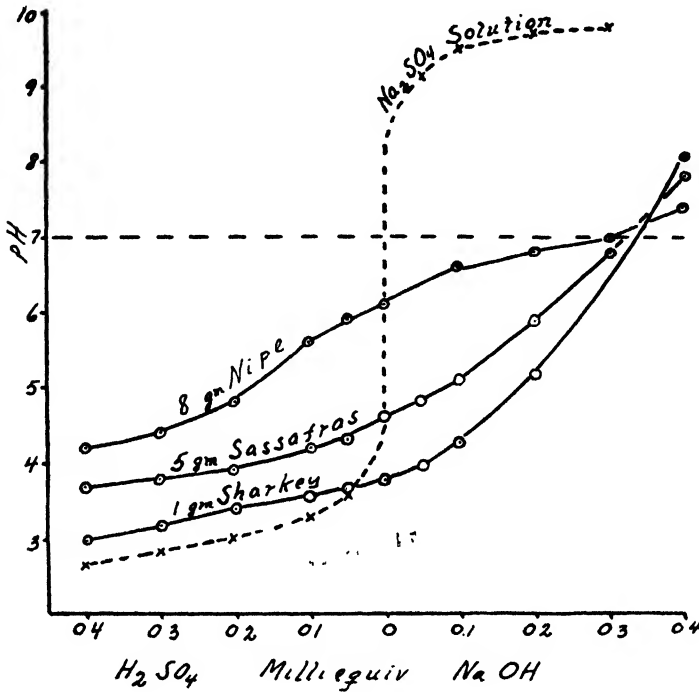


FIG. 30. THE NEUTRALIZATION CURVES OF THE THREE ELECTRODYALYZED SOILS IN FIGURE 28 WHEN TAKEN IN QUANTITIES OF EQUIVALENT BASE-BINDING POWER AT pH 7

TABLE 95

*The pH and carbon content of a Lakewood podzol and the major constituents of the extracted colloids*

HORIZON	WHOLE SOIL		EXTRACTED COLLOIDS			
	pH	Carbon	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>
		per cent	per cent	per cent	per cent	
A <sub>1</sub>	4.0	3.70	74.00	14.02	2.23	8.11
A <sub>2</sub>	4.2	0.27	79.40	14.09	2.06	8.78
B	4.65	1.23	45.40	35.81	11.69	1.78
C	5.15	0.09	45.60	21.67	10.15	2.74

quioxide ratios. This ratio is lowest in the B horizon where the sesquioxides have accumulated. The fact that the silica/sesquioxide ratio is higher in the C than in the B horizon although the pH is highest in the former must be due to the immature condition of the complex in the C horizon. As long as the

complex remains saturated to an appreciable extent with divalent cations, silica will be retained in excess of that quantity which is held by the sesquioxides alone. All unleached soil colloids possess a high silica content. The fact that the complex in B contains more humic acid in place of silica than the complex in the C horizon must also be considered.

The percentage of carbon indicates a high organic matter or humus content in the  $A_1$  horizon. It is also fairly high in the B horizon. The quantity of humic acids present in the complex is important when we consider the relationship between the silica/sesquioxide ratio and the amphoteric behavior of the complex. On the basis of this ratio the isoelectric pH should be in the following descending order

$$B > C > A_1 > A_2$$

but this order may obviously be modified by the presence of humic acid or any other acidoid and basoid. The silica/sesquioxide ratio does not then, even approximately, express the acidoid basoid ratio. Then it must again be emphasized that the ratio of silica to sesquioxides is a mass ratio and does not, therefore, express the activity of the acidoid and basoid residues. This will vary with the relative proportions between aluminum and iron, with the degree of hydrolysis, and with the dispersion of the material.

The chemical composition of the colloid does not in itself supply us with any adequate information concerning the nature of the complex.

Let us now see how the soil materials from the various horizons react. Figure 31 shows the neutralization curves of samples from the four horizons. Because of the sandy nature of the soil 10 gm. was taken in each case.

It will be noted that the samples from the A horizons whose colloids contain over 8 moles of silica to each mol of sesquioxide do not react amphotERICALLY to any definite degree. The slight exchange alkalinity developed by  $A_1$  at very low pH may be ascribed to the protein fraction of the organic matter in this material. The materials of the B and C horizons react, in strong contrast to that of the A horizons, definitely amphoteric. The pH of exchange neutrality in the  $Na_2SO_4$  solution is 5.0 in the B and 4.8 in the C horizon. The strength of the acid residue in the different complexes assumes the following order:

$$A_1 > A_2 > C > B$$

This is in agreement with the silica/sesquioxide ratios except in respect to the position of  $A_1$  and  $A_2$ . This discrepancy is of course largely due to the much higher humic content in the  $A_1$  horizon.

The influence of the humus expresses itself quantitatively more strongly than qualitatively, as will be seen by comparing the combining capacity of the  $A_1$  and  $A_2$  samples. Humic acids are no stronger acids than many of the mineral complexes having a high silica content (3, VI), but the combining capacity for bases is, because of the relatively small equivalent weight of humic acids,

considerably greater as compared to the mineral complex. Whereas 10 gm. of the  $A_2$  sample binds only about 0.15 m.e. NaOH at pH 7.0 we find by extrapolation that the  $A_1$  sample binds about 0.8 m.e. of the base.

The small combining capacity of the  $A_2$  sample as compared to the B and C samples is of course due to a smaller colloid content of the  $A_2$  horizon. The combining capacity per gram colloid would undoubtedly be greater in  $A_2$  than in B or C, since the cation exchange increases, within certain limits, with the silica sesquioxide ratio (3, V). Whereas the combining capacity of a soil sample varies with the colloid content the pH of exchange neutrality remains

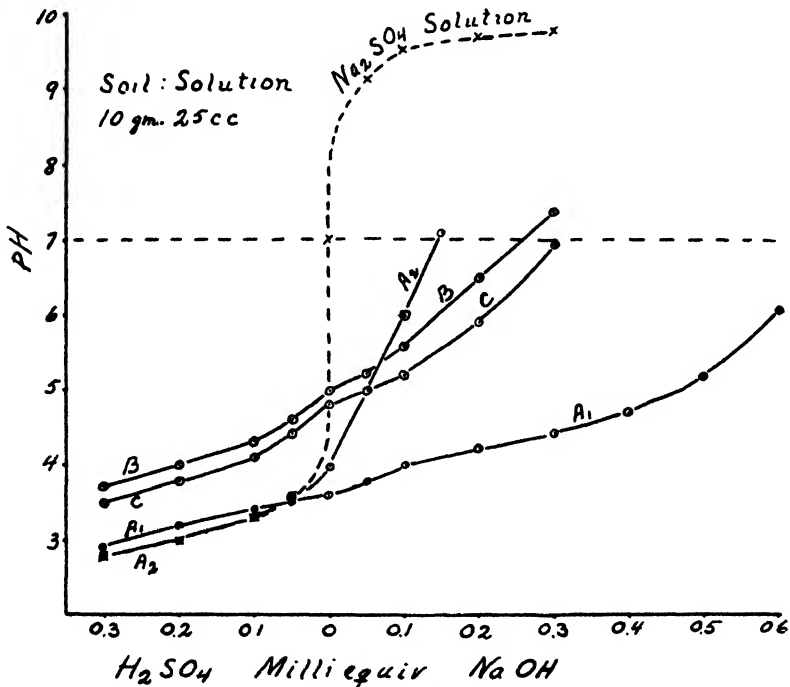


FIG. 31. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF A LAKEWOOD PODZOL PROFILE

unaffected, at least within wide limits. This is extremely important because it enables us not only to determine, by a very simple test, the amphoteric nature of the complex, but also, by comparing the exchange reactions of samples from the different horizons, to determine whether or not a soil is being podzolized.

The acid-oxalate extraction method proposed by Tamm for the study of podzolization has proved very useful (7, 2) but any method involving a chemical analysis is too time-consuming to be generally adopted. Furthermore, any method which is based upon the solvent action of a certain solution must always



remain more or less empirical. There are many other methods by which evidence of podzolization may be obtained. Some of these have recently been discussed by Joffe (1).

Since the soil colloidal complex reacts amphotERICALLY and since the chief result of podzolization consists in a change in the amphoteric nature of the complex according to a well-defined order, namely, a more acidic and less basic A horizon and a less acidic and more basic B horizon, it would seem that the most rational method is to be found in a determination of the amphoteric points of the respective complexes. The isoelectric points may be found cataphoretically, but this requires a costly apparatus. A determination of the ultimate pH or the pH of exchange neutrality is much more simple where no apparatus is available. Either method will yield the information sought. In the latter case, however, it is necessary that the soils be completely unsaturated, by electro-dialysis or otherwise. The pH of the soil in water represents then the ultimate pH whereas its pH in a neutral salt solution represents, very nearly, that pH at which the anions and cations combine with the soil complex in equal numbers.

In this work a normal  $\text{Na}_2\text{SO}_4$  solution was employed. It is possible that another salt and another concentration will prove more suitable. It is desirable to reduce the salt effect to a minimum, in view of which a more dilute solution might be chosen.

If the isoelectric point is to be determined by cataphoresis it is recommended that the pH be adjusted by HCl and that the soil be suspended in 0.01N, or less, NaCl. The presence of the salt greatly hastens the equilibrium so that the measurements can be made without serious errors the day after mixing.

#### THE REACTION OF AMPHOTERIC PRECIPITATES

In the following we shall present the neutralization curves of a number of the isoelectric precipitates discussed in the previous parts of this series (3, IX). The precipitates were prepared in large quantities in solutions less dilute than those used for the cataphoresis experiments referred to. They were then dried in a warm place, washed on the filter, and finally electro-dialyzed, dried, and analyzed. The composition is given in the figures.

#### *Aluminum and ferric "hydroxides" and "silicates"*

Figure 32 shows the neutralization curves and, at the points of intersection with the salt solution curve, the pH of exchange neutrality of aluminum "hydroxide" of three "silicates" of increasing degree of silication and of silica gel. Figure 33 gives the same values for the ferric complexes.

The figures require but little comment. Those who have read the preceding articles on isoelectric precipitates will recognize the same laws of amphoteric behavior: the displacement of the pH of exchange neutrality (as in the case of the isoelectric point) to the acid side and the increase in the cation (base) combining capacity as the silication of the complex is increased. Simultaneously

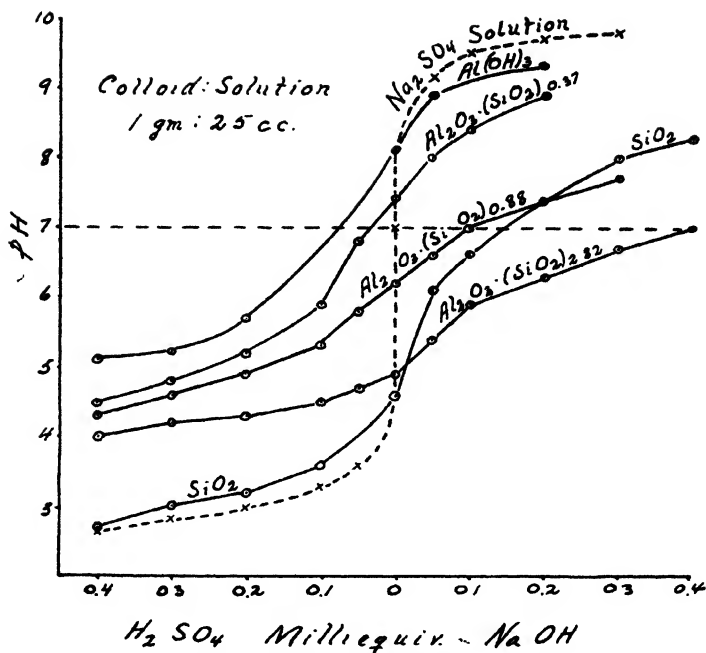


FIG. 32. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRO-DIALYZED ALUMINUM HYDROXIDE, SILICA GEL AND THREE ALUMINUM "SILICATES"

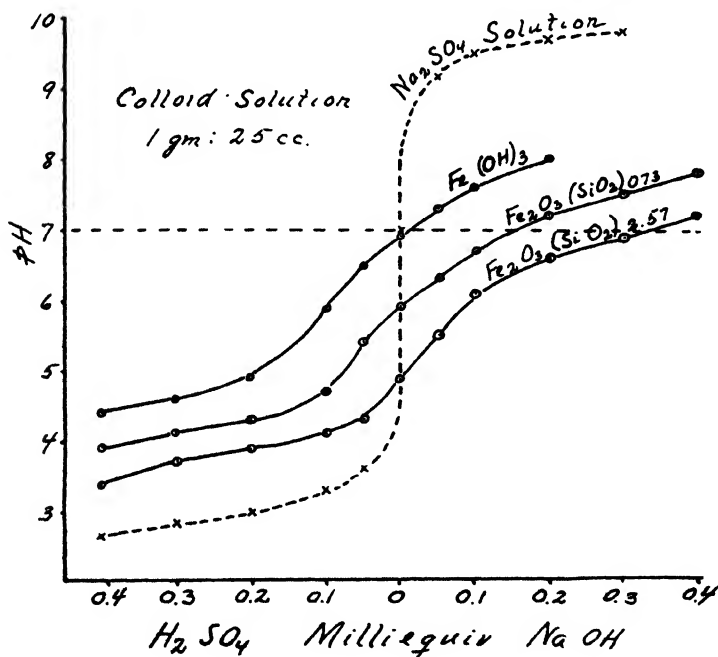


FIG. 33. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRO-DIALYZED FERRIC HYDROXIDE AND TWO FERRIC "SILICATES"

the complex loses its power to combine with anions (acids). The entrance of silicic acid into the complex strengthens the acid and weakens the basic residue. The fact that alumina is a stronger base than ferric oxide is brought out by the relative position of the amphoteric points.

The case of the silica gel is interesting in that it shows a lower cation combining power than the most highly silicated complex. This shows that the silicated sesquioxide complexes must attain a maximum in respect to the power to bind bases. This maximum has now been found and will be discussed in a later publication. The fact that the curve of the silica gel intersects the salt solution curve and thereby indicates a power to bind acid cannot be explained unless the basic ions were incompletely removed by electrodialysis. Silica may be amphoteric and bind acids at a very low pH but an exchange neutrality at pH 4.6 in  $\text{Na}_2\text{SO}_4$  would be highly anomalous in view of the behavior of the higher silicate complexes.

It will be noted that aluminum "hydroxide" together with the least silicated complex, both of which are stronger as bases than as acids and are therefore isoelectric above pH 7, yield an actual exchange alkalinity with the neutral salt solution just as the complexes whose acid residue is the strongest yield an actual exchange acidity. But even the latter type of substances yield, below the pH of exchange neutrality, an exchange alkalinity inasmuch as they cause a rise in pH. In a broad sense the term "exchange alkalinity" expresses therefore a rise in pH whereas the term "exchange acidity" expresses a lowering of the pH, no matter what side of the neutral point is involved.

### *The aluminum and ferric phosphates*

Figures 34 and 35 show the exchange-neutral points and the combining capacity of a few phosphated sesquioxides.

What has been said about the silicated complexes applies in general to the phosphated. But there are noteworthy differences. Thus the phosphate ion lowers the amphoteric points more, increases the cation combining capacity more, and suppresses the anion combining capacity more than the silicate ion. In other words, the phosphate ion leaves a relatively stronger acid residue and a weaker basic residue than the silicate ion. This is all in agreement with earlier observations (3, III).

If we compare the capacity of these precipitates to combine with bases as indicated by their neutralization curves, with their cation exchange capacity as reported in part V (3) it will be noted that the latter are appreciably greater. Although this might in part be ascribed to the use, in these experiments, of a more highly dissociable cation (Na), which therefore possesses a weaker displacing power, it is perhaps primarily due to the aged condition of the gels here used. The gels might have undergone considerable changes. Compounds of greater stability and lower reactivity might gradually be formed and crystal-

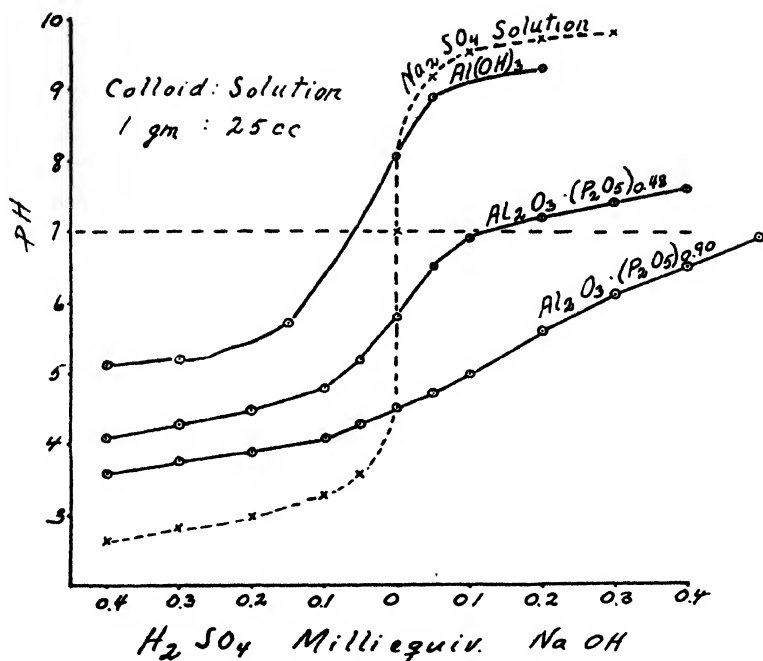


FIG. 34. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF TWO ELECTRODIALYZED ALUMINUM "PHOSPHATES" AND OF  $\text{Al}(\text{OH})_3$

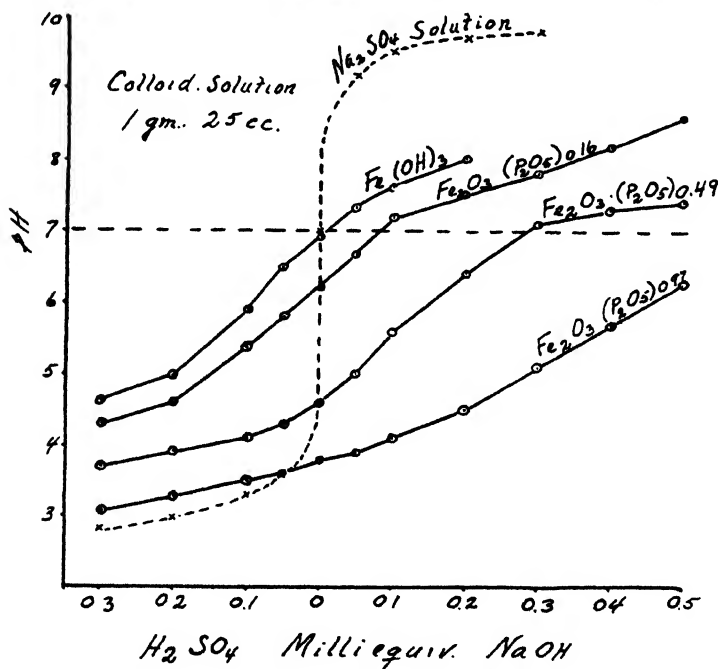
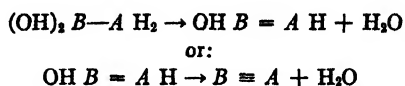


FIG. 35. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF THREE ELECTRODIALYZED FERRIC "PHOSPHATES" AND OF  $\text{Fe}(\text{OH})_3$

lized. The following might serve to illustrate the general nature of such a change:



all depending upon the relative stability.

The degree of the combination will depend upon the pH. At the isoelectric point where the ionization of the ampholytoid is at a minimum, there will be a maximum combination between the acidoid and the basoid constituents. Above and below this point there will be increasing hydrolysis resulting in a stronger acid and basic residue. Hence the combining power or exchange capacity of the colloid will be "built up" on the alkaline or acid side. The formation of more stable and less reactive compounds would therefore be favored by the electrolyzed, that is, by the uncombined, condition of the ampholytoid. In this way we might account for the loss of combining power of the electrolyzed and aged gels used in these experiments. Kaolin and other inactive and coarsely crystalline compounds may be assumed to be formed under conditions of unsaturation and a prevailing pH near that of the isoelectric point of the complex.

#### *Aluminum and ferric "humates"*

Figure 36 shows the exchange neutral points and the combining capacity of aluminum and ferric "humates" and of humic acid. The figures in the formulas  $\text{Al}_2\text{O}_3 \cdot (\text{Hum})_{0.265}$  and  $\text{Fe}_2\text{O}_3 \cdot (\text{Hum})_{0.277}$  express the gram weight of humic acid per millimol sesquioxide. These materials were prepared according to system 54 and 55 as shown in part IV (3). The "humates" were here used in the dry, powdered condition. Because of the irreversible, nondispersible nature of these materials, a considerable loss in combining power has resulted from the drying. The humic acid was used in the moist suspended form in which it has been kept. Because of the very much greater combining power of the latter only 0.25 gm. was taken for this experiment whereas the usual 1-gm. sample was taken in the case of each of the "humates."

The isoelectric pH values of the aluminum and ferric "humates" were 5.5 and 4.55 respectively. The pH of exchange neutrality in  $\text{Na}_2\text{SO}_4$ , as shown in figure 36, is 5.8 and 4.7 respectively. In spite of the considerable loss in combining power (see part V) the relative activity of the acid and basic residue seems therefore to have maintained itself. The capacity to bind base is also consistently greater in the ferric than in the aluminum complex. The ferric hydroxide, being a weaker base, leaves a greater as well as a more active acid residue.

Humic acid, as already noted (3, VI) reacts amphotERICALLY but its basic

group is weak and comes into play only at a low pH. Its pH of exchange neutrality in  $\text{Na}_2\text{SO}_4$  is about 3.6. The amphoteric nature of humic acid can be ascribed to the amino groups of the protein fraction of the complex.

Because of dispersion the humic acid curve could not be extended to the higher pH by the indicator method which was uniformly employed to compensate as much as possible for the salt effect.

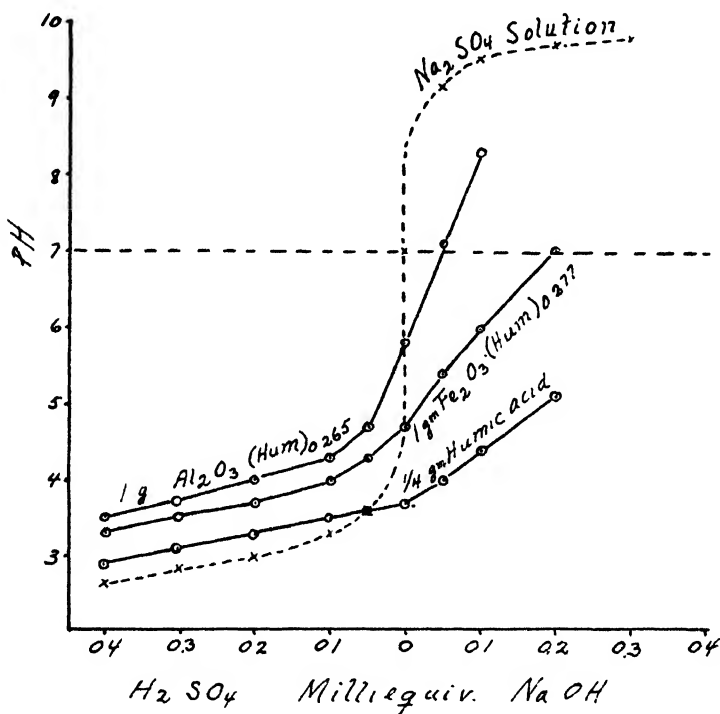


FIG. 36. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRODIALYZED ALUMINUM AND FERRIC "HUMATES" AND OF "HUMIC ACID"

Figures express gram humus per millimol sesquioxide

#### *Protein and protein "humates"*

The behavior of protein "humates" has already been discussed (3, VII). Figure 37 shows the neutralization curves of egg albumin, of humic acid, and of a mixture of both, each in 0.25-gm. quantities. The albumin was not electro-dialyzed. The point of intersection with the salt solution curve has therefore no definite relation to the isoelectric point.

The point of interest is the general form and position of the curves. The albumin combines with much more acid than the humic complex whereas the power to bind base is far greater in the latter. In a mixture of the two substances there results a complex which partly combines the properties of both.

The fact that the mixture, that is, albumin "humate," binds less acid than albumin alone and that, at the lower pH, it binds less base than the humic acid alone, proves that a mutual neutralization has taken place. At pH below the iso-electric point (about 4.8) of the albumin, where it plays the part of a base, it combines with humic acid forming a complex whose isoelectric point and combining capacity are intermediate to those of the individual components (3, VII). At pH above its isoelectric point the albumin combines primarily as an acid and must here add to the base binding power of the mixture. At high pH, the curve representing the mixture would undoubtedly cross the humic acid curve as indicated by the dotted extension of the curves.

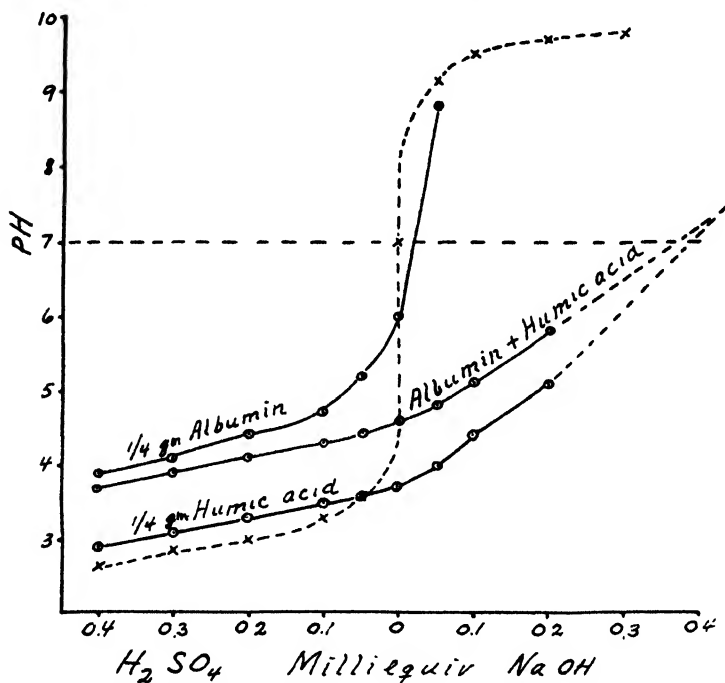


FIG. 37. THE NEUTRALIZATION CURVES OF ALBUMIN, OF "HUMIC ACID," AND OF ALBUMIN "HUMATE"

#### A CHEMICAL CLASSIFICATION OF SOILS

The most fundamental numerical values of acids and bases express their strength as defined by their dissociation constant and their combining capacity or equivalent weight. Substances which combine the properties of both acids and bases are in addition characterized by their isoelectric point. Since soil colloids react amphotERICALLY and possess, in various degrees, the properties of acids and bases it would seem that the most logical and scientific manner in which chemically to characterize and classify soils would be found in a numerical expression of their strength and capacity to react.

As already pointed out in part VI of this series (3), we cannot express the strength of a colloidal acid or base in the same terms, i.e., by the dissociation constant, by which we define the strength of the soluble acids and bases because we cannot apply the mass law to colloidal ionogens. But values such as the ultimate pH, the isoelectric pH, and the pH of exchange neutrality are all expressions of the strength of the acid and basic residues and, although these terms are no absolute measure of strength, they can be used and relied upon on a relative scale.

Since the ultimate pH and the pH of exchange neutrality represent the activity of the free, unsaturated ampholytoid (or acidoid) it is of course necessary that the soil be electrodyalyzed or otherwise unsaturated before these values are determined. The ultimate pH of a soil might be written  $pH_U$  whereas the pH of exchange neutrality might be designated  $pH_{U(KCl)}$  the formula within parenthesis denoting the salt solution employed. The U would distinguish these pH values from the corresponding values of the original, untreated soil.

When expressing the combining capacity or equivalent of a soil it is obviously necessary to state the pH at which the determination was made. Colloids contain displaceable H and OH of widely differing activity. As examples of very weak acids  $M''HPO_4$  and glucose may be mentioned, which both yield an acid dissociation constant as low as  $3.6 \times 10^{-13}$  (5). The H ions of these and other acids, of the same order, may not even begin to become displaced at a pH from 7 to 8.5, the usual upper limit in soils. (The degree of displacement at any pH will of course depend upon the dissociation of the salt formed.)

Very weakly acidic, basic, or amphoteric properties may be very generally associated with supposedly inert materials. It is conceivable that such properties depend entirely on the way a substance reacts with water, which contains the elements of both acids and bases. Cellulose is acidic as shown by its exchange reaction with KCl. The acidic nature of colloids appears to predominate. Michaelis (4) states that colloids divide themselves into acidoids and ampholytoids. No basoids are known. But it is only those acidoids which are active enough to become engaged within the usual range of soil reaction which are of importance in relation to the cation combining power of a soil. These acidoids comprise silicic acid and the silicated, humic acids and the humated, the proteins and the proteinated, and the phosphated complexes. To this list we might add titanium oxide. The sesquioxides are too weakly acidic appreciably to combine with bases below the usual maximum of soil pH. At high alkalinity a number of very weak acid groups come into play in addition to other stronger groups, set free by a hydrolytic cleavage of the complex. The "total" exchange capacity is therefore an indeterminable quantity.

As a measure of the base combining or exchange capacity of soils the amount of Ca adsorbed in equilibrium with  $CaCO_3$  would seem to be the most natural value. This value could also be obtained by carrying the neutralization to the corresponding pH. The important point is that soil investigators all agree upon a certain pH value and procedure for determining the combining capacity.



This capacity might be expressed by the symbols  $C_{(7.0)}$ ,  $C_{(8.0)}$ , or  $C_{(\text{CaCO}_3)}$ , the figures denoting the pH at which the capacity is determined or that the capacity was determined in equilibrium with  $\text{CaCO}_3$ .

The determination of the aforementioned values serves to define the soil in terms of its chemical character, qualitatively as well as quantitatively. Because of their intimate relationship to most of the properties of the soil, these values yield, in addition, a very general, if indirect, information about the soil and enable us, in a large measure, to interpret and to predict its behavior.

In addition to these values which must be looked upon as fundamental and, for long periods of time, fairly constant, we have to consider another value which is more subject to variation and which expresses the condition or degree of saturation of the soil complex. This value can be expressed numerically only with reference to a certain  $C$  value or to Hissink's  $T$  value. The degree of saturation or unsaturation need not be determined by a separate analytical method, as it is usually done, but may quite simply be computed from the neutralization curve of the electrodyalyzed soil as given in figures 28 to 31. The only additional information required is the pH of the soil in its original condition. The neutralization curve gives the combining capacity of the soil at any pH.

Figure 28 shows the combining capacities of the Sharkey soil (for Na ions in the presence of  $N \text{ Na}_2\text{SO}_4$ ) to be  $C_{(4.3)} = 0.1$ ,  $C_{(5.2)} = 0.2$ ,  $C_{(6.3)} = 0.3$ ,  $C_{(7.0)} = 0.345$  and  $C_{(8.0)} = 0.395$  m.e. per gram.

Assume now that the pH of the Sharkey soil is 6.3 in its natural condition. At this pH it would contain 0.3 m.e. exchangeable cations (we are here ignoring any errors resulting from a radically different set of conditions). At pH 7.0 the soil would contain 0.345 and at pH 8.0 it would contain 0.395 m.e. of cations other than H ions. The percentage saturation would be

$$\frac{C_{(6.3)} \times 100}{C_{(7.0)}} = \frac{0.3 \times 100}{0.345} = 87 \text{ per cent}$$

with reference to the combining capacity of the soil at pH 7.0, and

$$\frac{C_{(6.3)} \times 100}{C_{(8.0)}} = \frac{0.3 \times 100}{0.395} = 76 \text{ per cent}$$

with reference to a pH of 8.0.

Since most of the exchangeable cations consist of Ca ions, the most comparable results would be obtained by using  $\text{Ca}(\text{OH})_2 + \text{Ca Cl}_2$  instead of sodium. A thorough-going investigation would be desirable in order to determine the procedure most nearly applicable to natural conditions.

We thus see that practically all the important information dealing with the chemical nature of the soil may be obtained by the comparatively simple method of plotting the neutralization curve of the soil in its unsaturated condition. In a later publication we shall discuss the relationship between the am-

photeric nature of soils, as here brought out, and the point of injury to plant growth.

#### SUMMARY

The amphoteric nature of soils and their combining capacity are expressed in the form of neutralization curves obtained by treating the electrodyalyzed soil with a neutral salt solution to which free acid is added on the one side and free base on the other.

The pH developed in the neutral salt solution represents very nearly the pH of exchange neutrality, that is, the point at which the soil combines with an equal number of anions and cations of the salt. This point is related to the strength of the acid and basic residues of the soil complex. The ultimate pH is defined as the pH of the completely unsaturated soil ampholytoid. This also is strictly an intensity factor.

The combining capacity for anions and cations depends upon the quantity of the acid and basic residues, sufficiently active to become engaged at the pH in question.

Evidence of podzolization, which involves an alteration of the amphoteric complex by hydrolysis, may be found by applying the method to the soil profile.

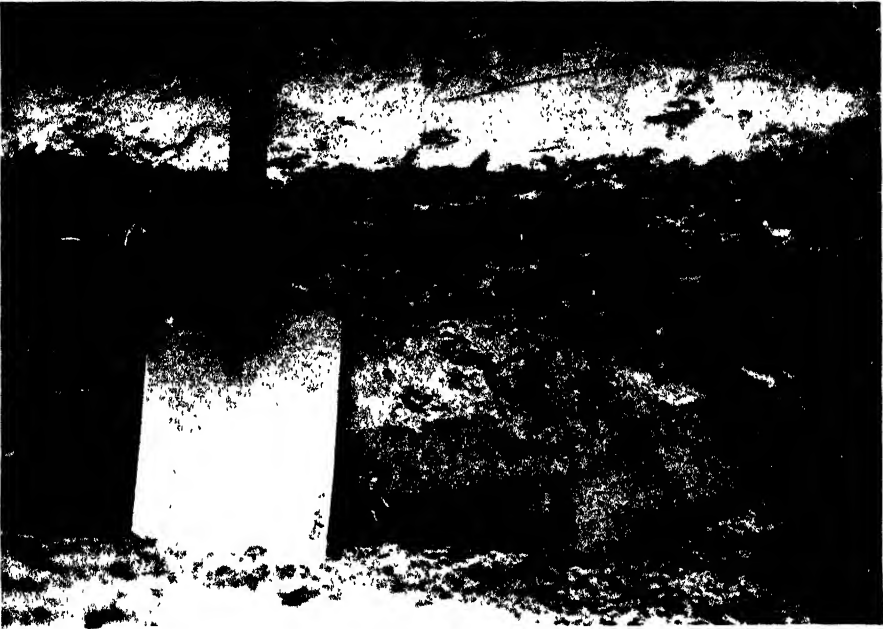
The colloid content and the saturation value may also be directly found from this type of curve.

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## PLATE 2

THE LAKEWOOD PODZOL PROFILE FROM WHICH THE SAMPLES WHOSE NEUTRALIZATION  
CURVES SHOWN IN FIGURE 31 WERE TAKEN





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